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16. ABSTRACT

The development of elaborate industrial societies has led to proliferation of a vast number-of complex chemicals for industrial, agricultural and domestic use. Some portion of these compounds eventually find their way into municipal and industrial wastewater. Unless specifically removed by waste treatment processes, they ultimately appear in receiving waters and water supplies, thus no longer is it sufficient to remove biochemical oxygen demand to protect the oxygen resource of the receiving water but individual organic compounds become a concern.

Knowledge of the toxicity and biodegradability of organic compounds will aid in designing wastewater treatment processes and be useful in elaborating the criteria for safe concentrations of organics in wastewaters discharged to surface waters.

This report describes the testing of twelve compounds both for biodegradability and toxicity. The compounds tested were: methylethyl ketone, dimethyl amine, dimethyl foramide, p-nitrophenol, o-chlorophenol, trichlorophenol, 2,2'dichlorodiethyl ether and five flourescent whitening agents used as components of household detergents. The biodegradation tests performed were respirometric measurements, river model and activated sludge model. Additionally, for some compounds supplementary tests were made for evaluation of their volatility, photolysis and adsorption on activated sludge. The toxicity was measured with use of fish Lebistes reticulatus & crustacean Daphnia magna.

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by

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FOREWORD

The Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our natural environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution, and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems for the prevention, treatment, and management of wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, for the preservation and treatment of public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research; a most vital communications link between the researcher and the user community.

Increasing numbers of products of commerce are reaching waste disposal facilities, and little is known concerning their effects on treatment. Some of these compounds are toxic, and others have profound physiological effects. The future dictates that all compounds reaching our surface waters should be biodegradable. Knowledge of what compounds that are not biodegradable will aid us in logically planning what changes we should make to eliminate them or develop treatment processes that will remove them.

This report presents the results of a testing program of several organic compounds for toxicity and treatability.

Francis T. Mayo
Director, Municipal Environmental
Research Laboratory

PREFACE

This project was conducted within the frame of the Marie Skłodowska Curie Fund, a bilateral monetary arrangement for cooperative scientific programs between Poland and USA.

This final report is a concise summary of four interim reports. The interim reports were not published but are on file in the libraries of Institute of Meteorology and Water Management, 01-673, Warsaw ul. Podlesna 61, Poland and U.S. Environmental Protection Agency, Environmental Research Center, Cincinnati, Ohio 45268. The interim reports, date and subject matter are:

Report #1, January, 1976 - MEK
Report #2, November, 1976 - MEK, DMF and DMA
Report #3, February, 1978, - PNP, OCP, TCP, and DCDEE
Report #4, June, 1979 - Five FWAs

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ABSTRACT

The biodegradability and toxicity of twelve organic compounds were investigated. They were:

methylethyl ketone (MEK)
dimethyl amine (DMA)
dimethyl formamide (DMF)
p-nitrophenol (PNP)
o-chlorophenol (OCP)
trichlorophenol (TCP)
dichlorodiethyl ether (DCDEE)
five various fluorescent whitening agents, all of the same stilbene-cyanuric type, but with different substitutes (FWA).

The investigations of the biodegradability were carried out by three methods, namely: (1) respirometric measurements, (2) tests in the river water, and (3) laboratory activated sludge units.

MEK, DMA, DMF, PNP, OCP, TCP were all biodegradable. DCDEE was found to be a biologically inert substance under conditions of the tests. DCDEE is a volatile substance; therefore, it may be partially blown off in the aeration chamber. The FWA's are fairly resistant to biodegradation; however, they do not affect the overall treatability of wastewater.

Long term respirometric measurements using a "Sapromat" that prints out the amount of oxygen uptake every hour was very useful in supplying data for kinetic parameters and inhibiting effects of various compounds. The activated sludge tests gave overall treatability information and time required for acclimatization.

The toxicities of the test substances were determined by bioassays using fish Lebistes reticulatus and Daphnia magna. The toxicity of MEK, DMF, TCP are low while PNP, OCP, TCP were fairly high to fish. The FWA's varied from low to high toxicity to fish depending on the molecular structure.

The work has been accomplished within the Polish American agreement Project PL-480, Grant PR-05-532-15 by the Institute of Meteorology and Water Management, Department of Water Chemistry and Biology at Warsaw, Poland, for the U.S. Environmental Protection Agency.

The work was finished in 1979.

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SYMBOLS

a - Henry's coefficient of the equilibrium between volatile substance content in the liquid and gaseous phase

a and b - Langmuir's isotherm coefficients

c - concentration of tested compound, mg/l

c_e - - tested substance effluent concentration, mg/l

c_o - initial concentration or the inflow concentration, mg/l

k_n - kinetic coefficient of the nutrient medium biodegradation, decimal day^{-1}

k_s - kinetic coefficient of the tested compound biodegradation, decimal day^{-1}

K_{ad} - the coefficient of the FWAs removal by the adsorption and removal with excess sludge $g^{-1} \cdot l \cdot h^{-1}$

K_b - the coefficient of the FWAs removal due to the biodegradation $g^{-1} \cdot l \cdot h^{-1}$

K_{COD} - overall treatability coefficient, being the COD decrease in the activated sludge unit per 1 mg/l COD in the effluent per 1 g/l MLSS and per 1 hour of retention time, $g^{-1} \cdot l \cdot h^{-1}$

K_{ph} - the coefficient of the FWAs removal by photolytic decomposition, $g^{-1} \cdot l \cdot h^{-1}$

K_s - the tested substance removal coefficient, being the decrease of the tested substance contents in the activated sludge unit per 1 mg/l in the effluent per 1 g/l MLSS and per 1 hour of retention time, $g^{-1} \cdot l \cdot h^{-1}$

L - the BOD, remaining at the time t, mg/l O_2

L_c - the final carbonaceous BOD, mg/l O_2

L_u - the unit oxygen demand, caused by 1 mg of the given substance, mg O_2/mg substance

m - MLSS, dry mass, g/l

N - NH_3 content at the time t, mg/l NH_3-N

N_f - final NH_3 contents, mg/l NH_3-N

NOD - the oxygen demand caused by nitrification, mg O_2/l

R - inhibition effect (the ratio of treatability coefficient in the test unit K_{COD_t} and control unit K_{COD_c})

$r = \frac{k_s}{k_n}$ - The relative rate of the tested substance biodegradation (in the monomolecular reaction phase), dimensionless

t_o - lag phase of the tested substance biodegradation in river water

t_1 - time needed by activated sludge to restore normal treatment of wastewaters background in the presence of the tested substance

t_2 - time needed by activated sludge to decompose the tested substance

Δt_1 - inhibition time of nutrient medium BOD in the presence of the tested substance, days

Δt_2 - inhibition time of the tested substance BOD, days

v - the biodegradation rate in river water in the phase of constant rate, mg/l·h

y - BOD at the time t, mg/l O_2

t_r - the retention time, hours

Θ - the sludge age, days

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Technical contributions to these studies were made by technical staff of the Department of Water Chemistry and Biology of the Institute of Meteorology and Water Management. Their assistance is sincerely appreciated.

SECTION 1

INTRODUCTION

The development of elaborate industrial societies in the United States and in Europe, has led to proliferation of a vast number of complex chemicals for industrial, agricultural and domestic use. Some portion of these compounds eventually find their way into municipal and industrial wastewaters. Unless specifically removed by waste treatment processes, they ultimately appear in receiving waters and water supplies, thus no longer is it sufficient to remove biochemical oxygen demand to protect the oxygen resource of the receiving water but individual organic compounds become a concern.

Along with the benefits of chemicals there are risks. Unfortunately, exposure to some chemicals, sometimes in very small amounts, can lead to tragic and irreversible biological effects including cancer (carcinogenesis), transmissible genetic damage (mutagenesis), and birth defects (teratogenesis). The negative effects of toxic organic substances are greater when the substances are resistant to biodegradability. New organic compounds introduced into the water environment should be biodegradable, easily removed in treatment and their residual easily degraded in water bodies. Knowledge of the toxicity and biodegradability of organic compounds will aid in designing wastewater treatment processes.

This report describes the testing of twelve compounds both for biodegradability and toxicity. The compounds tested were: methylethyl ketone (MEK), dimethyl amine (DMA), dimethyl foramide (DMF), p-nitrophenol (PNP), o-chlorophenol (OCP), trichlorophenol (TCP), 2,2'-dichlorodiethyl ether (DCDEE) and five flourescent whitening agents (FWA) used as components of household detergents.

SECTION 2

CONCLUSIONS

Biodegradation and toxicity of twelve organic compounds were studied. The biodegradation tests performed were respirometric measurements, river model, and activated sludge model. Additionally, for some compounds supplementary tests were made for evaluation of their volatility, photolysis and adsorption on activated sludge. The toxicity was measured with use of fish Lebistes reticulatus and crustacean Daphnia magna.

The obtained data permitted the calculation of several parameters characterizing the properties of tested compounds. From the respirometric data, the unit oxygen uptake L_u caused by 1 mg of a given substance, was calculated. The processing of BOD curves parameters gave three kinetic characteristics: the time Δt_1 of the inhibition of the standard medium decomposition (yeast extract or glucose), the time Δt_2 of the inhibition of the tested substance decomposition, and the ratio $r = k_s/k_n$ of the Streeter-Phelps coefficients corresponding with the tested substance decomposition k_s and standard medium decomposition k_n. This parameter indicates, whether the tested substance biodegradation is faster $r > 1$ or slower $r < 1$ than the biodegradation of the standard medium.

The river model tests indicate the lag phase duration t_o, which is the time of acclimatization of river water biocenosis. The same data permitted the calculation of the rate of the tested substance decomposition v, mg/l·h, during the phase of the constant rate of the decomposition.

The tests with the activated sludge supplied data on the acclimatization time t_1, after which the activated sludge normally decomposes the wastewaters background, and t_2, after which also the tested substance is decomposed. The treatability of wastewaters containing the tested substance was characterized by means of K coefficients, one referring to the tested substance removal K_s and the second one referring to the overall treatment K_{COD} measured as COD decrease. Both coefficients are the decrease of tested substance or of the COD, calculated per 1 mg/l in the effluent, and per 1 g/l MLSS and per 1 hour of retention time. The inhibiting effect of the tested substance on the overall treatment of wastewaters was characterized by the ratio R of K_{COD} values observed in the test unit and in the control unit.

The results of the tests are summarized in Tables 1 - 5.

TABLE 1. THE RESPIROMETRIC DATA

Substance	Concentration test range	Measured unit oxygen demand, L_u	Kinetic parameters at the concentration c_o			
			c_o	Δt_1	Δt_2	r
	mg/l	mg O_2/mg subst.	mg/l	days	days	
MEK	50 - 800	2.12	50	0.5	0.5	1
			800	1.0	1.0	0.2
DMA	40 - 900	2.13	300	0	5	0.75
			450	0	> 13	0
DMF	80 - 440	1.40	80	0	3	0.8
			440	0	4.5	0.8
PNP	10 - 100	1.30	50	0	0.8	1.5
			100	4	> 15	0
OCP	5 - 1200	0	10	0	2	0.3
			200	0.5	> 11	0
			1200	> 11	> 11	0
TCP	10 - 540	0.9	12	0	5	n.d[xx]
			90	0	> 18	0
DCDEE	0.7 - 500	0	0.7	0	7	0
			500	0	> 16	0
FWAs[x]	100	0	100	0		

[x] The tests with all FWAs gave the same results. The determinations of Δt_2 and r were not possible on the basis of respirometric data because of the lack of the oxygen demand, despite the partial FWAs decomposition.

[xx] not determined.

TABLE 2. THE DECOMPOSITION OF SUBSTANCES IN THE RIVER WATER

Substance	Concentration	Kinetic parameters, biocenosis not acclimatized		Kinetic parameters, biocenosis previously acclimatized	
		t_o	v	t_o	v
	mg/l	days	mg/l·h	days	mg/l·h
MEK	20	0.8	0.6	0	1.2
DMA	22	1.3	0.4	0.8	0.55
DMF	28	2	0.55	0	0.8
PNP	5	0	0.1	0	~ 0.3
	20	1.4	0.35	0	~ 0.5
OCP	2	13	0.02	0	0.03
	20	22	0.10	0	0.10
TCP	5	6.5	0.13	0	0.20
	15	5	0.27	2	0.38
DCDEE	3	> 18	0	-	-
	7	> 18	0		
FWAs[x]	10	> 30	0	-	-

[x] In the absence of the light.

TABLE 3. PHOTOLYTIC FWAs DECOMPOSITION IN WATER
(LIGHT INTENSITY 1000 LUX)

Substance	Concentration mg/l	Rate of photolysis in river water, mg/l·h	Rate of photolysis in distilled water, mg/l·h
FWA-1	10	5	7
	50	6	11
FWA-2	10	9	14
	50	13	13
FWA-3	10	1.5	5
	50	5	5
FWA-4	10	2	8
	50	4	9
FWA-5	10	5	11
	50	7	12

TABLE 4. TREATABILITY OF THE TESTED SUBSTANCE BY ACTIVATED SLUDGE

Substance	c_o mg/l	t_1 days	t_2 days	K_s $g^{-1}l \cdot h^{-1}$	R
MEK	200	0	8	high	1
	400	0	9	high	1
DMA	20	0	6	high	~ 1
	135	14	12	high	~ 0.7
DMF	70	38	38	high	~ 1
PNP	5	0	~ 15	high	~ 1
	200	0	3[x]	0.7	1
OCP	5	0	6	0.2	1
	200	-	-	0	0
TCP	5	0	0	high	1
	25	-	-	0	0
DCDEE	5	0	-	0	1
	10	0	-	0	1
FWA-1	10	0	0	2.1	1
	40	0	0	2.5	~ 1
FWA-2	6	0	-	0	~ 1
	200	0	-	0	0.7
FWA-3	5	0	0	0.03	~ 1
	80	0	0	0.01	0.3
FWA-4	4	0	-	0	~ 1
	44	0	-	0	~ 1
FWA-5	4	0	-	0	~ 1
	40	0	-	0	~ 1

[x]the value is the additional time needed by the sludge previously acclimatized to the presence of 40 mg/l PNP.

TABLE 5. LETHAL CONCENTRATIONS OF THE TESTED SUBSTANCES TO *Lebistes reticulatus* AND *Daphnia magna*

Substance	LC_{50}- 96 h to *Lebistes reticulatus* mg/l	LC_{50}- 24 h to *Daphnia magna* mg/l
MEK	5700[XX]	n.t[X]
DMA	60[XX]	"
DMF	1300[XX]	"
PNP	19	"
OCP	12	"
TCP	4.5	"
DCDEE	190	"
FWA-1	16	22
FWA-2	1800	3900
FWA-3	110	surpasses solubility
FWA-4	3000	9400
FWA-5	6400	5600

[X] not tested

[XX] LC_{50}- 24 h

The obtained data lead to the following conclusions:

MEK is easily biodegradable. The respirometric data show that MEK practically does not inhibit the microbiological activity and is biodegraded at its initial concentration up to 800 mg/l. At the high concentrations, the biodegradation rate is lower. The river model tests and treatability tests confirm that MEK is easily biodegraded and does not affect the overall treatability. The toxicity of MEK is low, its LC_{50} for fish Lebistes is near 6 g/l. This is much more than the value, which could be expected in the wastewaters.

DMA, is easily biodegradable at the concentrations not surpassing 300 mg/l. The biodegradation rate is rather high, and the time needed for microbiological acclimatization is short. In the treatment by activated sludge, DMA is easily removed from wastewaters and has little effect on the

overall treatability of wastewaters. The toxicity of DMA is high. A concentration of 60 mg/l of DMA causes 50 percent mortality of fish.

DMF is easily biodegraded. Its unit oxygen demand and unit amount of NH_3 involved in the biodegradation are not very high. The DMF biodegradation in the rivers and its removal by means of activated sludge needs a period for microorganisms acclimatization. The rate of DMF removal by acclimatized sludge is high, and no harmful affect of DMF on the overall treatability occurred. The toxicity of DMF is low. The value of LC_{50}- h for fish is above 1 g/l.

PNP is biodegradable at concentrations up to 50 mg/l. At the higher concentrations, 100 mg/l and above, PNP is toxic and biodegradation does not occur. In the river water at concentrations up to 20 mg/l, PNP is biodegraded at a rather high rate. The activated sludge removes PNP from wastewaters with high efficiency. Also the overall efficiency of wastewater treatments is not affected by the PNP presence. PNP is highly toxic to higher animals; its LC_{50}- 96 h is ca. 20 mg/l.

OCP is biodegradable only at low concentrations up to 10 mg/l. At concentrations up to 200 mg/l OCP is not biodegraded, but it does not inhibit significantly the other biochemical processes. OCP concentration of 1200 mg/l causes the poisoning of the microorganisms. OCP biodegradation in the river water needs a long period of acclimatization, lasting 2 to 3 weeks. Even after this period, the biodegradation rate is low. The activated sludge removes OCP with low efficiency. The overall treatability is not affected by 50 mg/l OCP, but 100 mg/l OCP causes the poisoning of the activated sludge. The toxicity of OCP to fish is high, LC_{50}- 96 h being equal to 12 mg/l.

TCP is biodegradable at concentrations up to 35 mg/l, but needs a certain period for microorganisms acclimatization. The TCP biodegradation in river water proceeds at a rather high rate. The activated sludge removes TCP from wastewaters containing up to 18 mg/l. This TCP content does not affect the overall efficiency of the wastewater treatment, but 25 mg/l causes poisoning of the sludge, and both TCP and COD removal drop down to zero. TCP is very toxic to higher animals. Its LC_{50}- 96 h to fish amounts to ca. 5 mg/l.

DCDEE is a biologically inert substance. No DCDEE biodegradation was observed, even at a low concentration of 0.7 mg/l. DCDEE does not affect the microbiological activity, even at concentrations as high as 500 mg/l. The only effect observed was the inhibition of the nitrification partially occurring at 3 mg/l DCDEE. The river model and results obtained with use of the activated sludge model confirm these conclusions. DCDEE is a volatile substance and may be partially blown off the wastewater in the aeration chamber. The LC_{50}- 96 h for fish is 190 mg/l.

FWAs do not cause any oxygen demand but they are partially decomposed at the conditions of the respirometric tests. All FWAs are quite resistant in the river water in the absence of light. Illumination of the FWAs solutions causes their decomposition at a high rate. Both in the

respirometric and the river water tests, the FWAs decomposition consist most probably in transfiguration of the molecules or in their breaking into large fragments.

In the treatment by the activated sludge, FWAs were resistant, except FWA-1 which was biodegraded. FWA-3 was partially removed, but at a very low rate. FWAs do no affect the overall treatability of wastewater until their contents surpass 80 mg/l. The results of the bioassays show that FWA-1 is the most toxic, the toxicity of FWA-3 is moderate and the toxicities of other FWAs are low.

SECTION 3

RECOMMENDATIONS

The biodegradation study of organic compounds in the respirometer, in the river water model and in the activated sludge model gives useful information on the condition of organic compounds removal in the treatment plant and on their fate in river water. Those data supplemented by the toxicity data can be useful in elaborating the criteria for the safe concentration of organics in the discharged wastewater and the receiving water.

Based on the obtained results of the study, the general recommendations formulated are:

MEK is a substance of weak toxicity and is easily biodegraded, therefore its harmfulness to the environment is limited. However, the BOD of MEK solutions is high and may cause oxygen deficiency in the rivers polluted with MEK or extensive BOD_5 load in the wastewater. MEK does not contain heteroatoms in the molecules, therefore the biodegradation of the high MEK contents will require the addition of mineral nutrients.

DMA is moderately toxic and easily biodegraded. The high BOD and the ammonia development in the DMA biodegradation process may cause, in rivers polluted with DMA, an oxygen deficiency and an increase in ammonia.

DMF is weakly toxic and easily biodegraded by acclimatized microorganisms. The treatment of wastewater, containing DMF, by means of the activated sludge needs the acclimatization of the sludge to the presence of DMF.

PNP is a highly toxic substance. PNP biodegradation occurs in river water at low concentrations. The wastewater containing PNP can be treated by activated sludge after sludge acclimatization.

OCP is a highly toxic substance. OCP is biodegraded at low concentrations in the river water and in the activated sludge process, but at a low rate. Long retention times might be needed.

TCP is the most toxic of all substances tested. The TCP biodegradation proceeds at low concentrations. It is suggested that severe limitation be placed on TCP discharge into sewage systems and into rivers.

DCDEE is moderately toxic, but is a biologically resistant substance. Its presence in the wastewater does not affect treatability, but DCDEE is not removed except by the partial blowing off with the air in the aeration basin. Special treatment may be needed.

FWAs are weakly toxic except highly toxic FWA-1 and moderately toxic FWA-3. In the treatment process FWAs are resistant, except FWA-1 which is removed at a high rate and FWA-3 which is removed slowly. In the natural environment, FWAs are easily decomposed due to photolysis but this process has not led to FWAs complete decomposition. Rather, photolysis causes FWAs molecules to break into large fragments.

Not enough compounds were examined to form any conclusions as to any relationship between chemical structure and biodegradability. It is recommended that such be done. It will not be possible to test all the organic compounds now found in wastewater and all the new ones that will be developed in the future. Some chemical, physical or structural property or a combination of several parameters must be found that would lead to the prediction of the behavior of organic compounds in wastewater treatment plants and surface waters.

SECTION 4

BACKGROUND INFORMATION

Methylethyl ketone $CH_3.CO.C_2H_5$ is a liquid boiling at 81 °C; one liter of water dissolves 292 g of MEK at 20 °C. MEK is used in industry as a solvent. MEK is found in some industrial wastes (e.g., in the waste from production of synthetic leather Corfam, paints and lacquers).

MEK in concentrations up to 50 mg/l does not influence the biochemical processes (67).

According to Turnbull (61), the TL_m for fish Lepomis macrochirus is 5640 mg/l and a noticeable influence on these fish was found at 3380 mg/l. TL_m for fish Gambusia affinis was found to be 5600 mg/l (64). Bringmann and Meinck (9) determined the toxic concentration of MEK for bacteria Pseudomonas as 2.5 g/l, for algae Scenedesmus as 12.5 g/l, and for protozoa Colpoda as 5 g/l. The experiments performed by Wlodek (70) have shown that LC_{50}- 48 h for Asellus aquaticus is 2850 mg/l. Wlodek proposes that the permissible, safe concentration is 28.5 mg/l.

The following methods of MEK determination are known:

- Gas chromatographic method (after distillation with butyl alcohol). The limit of detection is equal to 0.004 mg/l (12).

- Spectrophotometric method based on the reaction of MEK with vanillaldehyde. The developed color is measured at 600 nm (62).

- Redox titration with the iodine in alkali solution (59).

- Amperometric titration with hydroxylamine hydrochloride in a waterless solution of pyridine and isopropyl alcohol (7).

Dimethyl amine $CH_3.NH.CH_3$ is a gas condensing at 7 °C. DMA is easily dissolved in water, forming an alkali solution. DMA is found in some industrial waste (e.g., from the production of synthetic leather Corfam). It is used in the synthesis of many substances.

The toxic influence of DMA on higher organized life is more marked than on lower organisms. Toxic concentration for fish Leuciscus cephalus is 30 - 50 mg/l (19) whereas for algae Scenedesmus and for protozoa Colpoda it is 250 mg/l. A toxic influence was found for bacteria Pseudomonas at the concentration 1 g/l (38). Corti (13) observed that DMA in concentration of 205 mg/l influences fish Salmo irideus after 33 - 40 sec and death

occurred after 10 - 13 min. Water Quality Criteria (66) states 0.7 mg/l DMA changes the taste of the fish meat of trout. Dzhanashvili (15) proposed the value of permissible concentration of DMA as 0.1 mg/l.

DMA is determined usually by GC method (17), (30), (41). Kubelka (30) set the detection limit of DMA in water as 0.01 mg/l. Other methods of DMA determination in water are as follows:

- Spectrophotometric method with carbon disulfide and ammonia solution of cupric acetate (10), (52).

- Fluorometric method by the exciting with light of 365 nm and by the measurement of the emitted fluorescent light at 535 nm (65). The samples are prepared by use of the thin layer chromatography.

- Polarographic method (1).

Dimethyl formamide $(CH_3)_2N.CHO$ is a liquid boiling at 150 °C. DMF is soluble in water in any ratio. DMF is used in chemical industry as solvent. It occurs in wastewater from the production of synthetic leather Corfam.

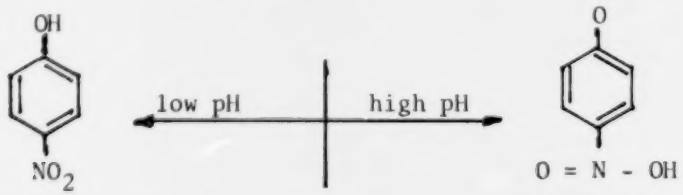
According to Zamyslova and Smirnova (74) DMF in concentration 10 - 500 mg/l does not change the BOD of water and wastes. High amounts of DMF in water causes an increase of ammonia. Permissible concentration of DMF in receiving water in the Soviet Union is 10 mg/l.

The bioassays of DMF with Asellus aquaticus (73) indicated that LC_{50}-48 h is equal to 9628 mg/l.

DMF contents in water is usually determined by the gas chromatographic method (30), (34).

The spectrophotometric method is based on the reaction of DMF with picric acid forming a yellow colored complex (11).

p-Nitrophenol is a solid compound, melting at 114 °C and boiling at 279 °C, sublimating at a temperature below b.p. Depending on pH, PNP may exist in two tautomeric forms: the benzene or quinone form:



benzene form quinone form

PNP is used for wood impregnation. It is used also as raw material in production of pesticides and azo dyes.

Biodegradation of PNP was studied by Pitter (47), (48). Comparative investigation of various nitro phenols showed that biodegradation decreases

with the increase of nitro groups on the molecule. The highest rate of biodegradation was shown by the nitrophenols with the nitro group in the para position. Pitter (48) measured the rate of PNP biodegradation with use of COD determination and found it equal to 17.5 mg COD/g·h.

The following methods of PNP determination are known:

- Spectrophotometric method based on reduction of nitro groups to amine groups and by diazotization, the dye is developed and measured (44).

- Spectrophotometric method based on development of yellow colored sodium pseudosalt of nitrophenol in presence of sodium hydroxide (4), (45), (56).

- Polarographic measurements in the presence of methanol and sodium hydroxide made at $E_{1/2}$ = 0.98 - 1.05 V (73).

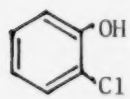
- Gas chromatographic method (8), (26), (55).

- Thin layer chromatographic method (40), (42), (58).

- Phosphorescence method (37).

- Atomic absorption spectrophotometry (AAS) method based on the creation of a complex compound of PNP with cobalt and determination of cobalt by AAS method (39).

o-Chlorophenol is a liquid melting at 9 °C and boiling at 175 °C. The solubility in water at 20 °C is equal to 28 g/l. OCP has very strong antiseptic properties. It is used in production of antiseptic agents and pesticides. Additionally OCP can be developed in water during the chlorination process.



Ingols (22), (23), (24) performed an extensive study on the degradation of chlorophenols by means of activated sludge. He found that the persistence of chlorophenols increases with the increase of the number of chlorine atoms on the molecule and depends on the isomeric position of those atoms in the aromatic ring. It was found that OCP in concentration 100 mg/l was fully degraded within 3 days. Pitter (47), (48) found the rate of OCP biodegradation by activated sludge, determined on the basis of COD measurement, was equal to 25 mg COD per hour and per 1 g of activated sludge.

Lammering and Burbank (31) found that the introduction of chlorine into phenol ring increases the toxicity. The toxicity of chlorophenols increases with the increase of the number of chlorine atoms in the phenol ring (13), (63), (66). Ingols (22), (23) found that TL_m at 23 °C for fish is equal to 58 mg/l.

OCP content can be determined by the following methods:

- Spectrophotometric method based on the formation of the indophenol dyes in the reaction of phenols with 4-aminoantipyrine (21), (53), (56). It is the method used most commonly for determination of phenols.

- Spectrophotometric method based on reaction of chlorophenols with diazotizated p-nitroamine and magnesium ions (72), (32).

- Titrimetric method based on bromination or chlorination of aromatic ring and iodometric titration of the excess oxidation agents (3), (29), (49).

- Gas chromatographic method (2), (27), (46).

Trichlorophenol is a solid compound, melting at 67 °C and boiling at 244 °C and soluble in water in the amount of 0.8 g/l at 20 °C. The use of TCP and occurrence in wastes and water is similar to OCP.

Biodegradation of TCP was studied by Ingols (23). In the tests lasting two days TCP was totally degraded at a concentration of 50 mg/l, but at a concentration of 400 mg/l TCP, the biodegradation was completely inhibited. According to Ingols (23) the TL_m at 25 °C for fish is equal to 3.2 mg/l.

TCP can be determined by the following methods:

- Spectrophotometric method based on the formation of indophenol dyes in reaction of phenols with 4-aminoantipyrine (2), (53), (69).

- Thin layer chromatographic method (68), (75).

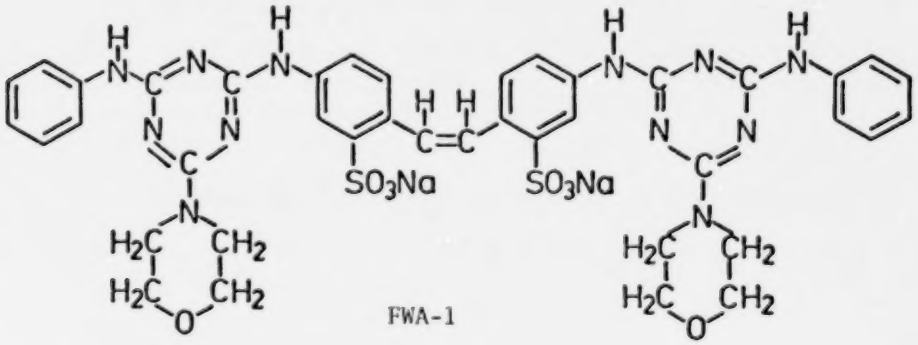
- Gas chromatography (20), (43), (51), (50).

2,2'-dichlorodiethyl ether $CH_2Cl.CH_2O.CH_2.CH_2Cl$ is a colorless liquid boiling at 178 °C. The solubility in water is 1 g/l at 20 °C. DCDEE is used as an insecticide, as cleaning agents and as a solvent. It is a byproduct in the production of the synthetic fibers, glycoles, antifreezing agents and some pesticides (36). Meager data exist on toxicity of DCDEE (60).

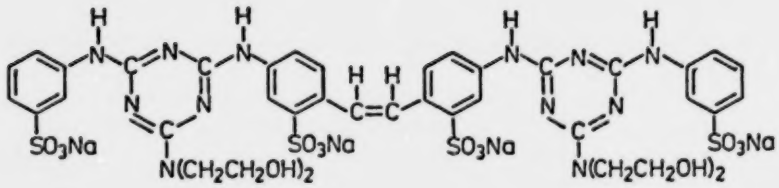
DCDEE is determined in water mostly by gas chromatography (14), (28) and gas chromatography-mass spectrometry method (6).

Fluorescent whitening agents are used for the improvement of the color of the textiles, paper, plastics and other materials. They are added to detergents used in households. FWAs are solid compounds, soluble in water in the range 0.5 - 100 g/l depending on the kind of FWA. In the frame of the present work five commercial FWAs were studied. The samples contained 8 - 30% of the sodium chloride.

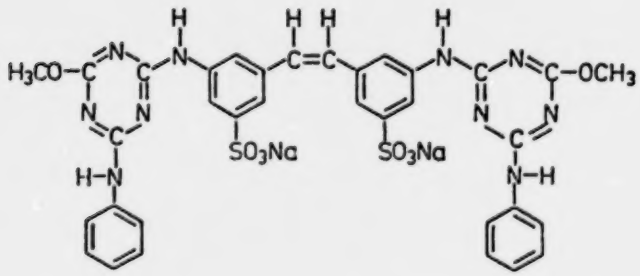
The structural formulas of five tested FWAs are given below:



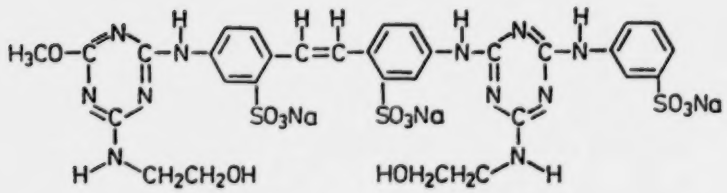
FWA-1



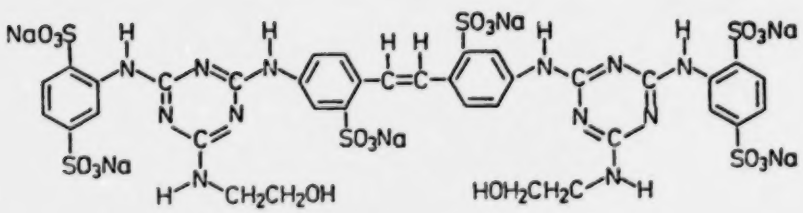
FWA - 2



FWA - 3



FWA - 4



FWA - 5

Jensen (25) found that FWA can accumulate in fish. He analysed various species of fish taken from rivers in Sweden and found measurable quantitites of FWA in the muscle of fish. When fish were returned to water not containing any whitener, they lost the FWAs very quickly (57).

The study of toxicity of FWAs for trout showed that LC_{50}- 96 h ranged from 108 - 1780 mg/l (76).

The most common method for determination of the FWA in water is fluorimetric method (16), (18), (33). The mercury lamp is used for excitation with the wavelength of 370 nm, and the fluorescence is measured at 440 - 480 nm. Thin layer chromatography method is used also for determination of FWA.

There are some advantages to the combination of the two above mentioned methods, i.e. the separation of the FWA by use of thin layer chromatography and the identification by means of fluorimetric measurement (18). The combined technique of TLC with radiometric measurement and with IR-spectrophotometry is also known (54).

SECTION 5

EXPERIMENTAL PROCEDURES

RESPIROMETRIC MEASUREMENTS

Apparatus

The BOD tests were carried out by means of "Sapromat" respirometer. The principle of measurement is the following: The sample of solution, in which the BOD is to be measured, is put into the 0.5 l flask in the amount of 332 ml. The flask is then hermetically sealed. The oxygen uptake in the solution causes a reduction of the pressure in the air space in the flask which switches on the electrolytic oxygen generator.

The automatic counter records the amount of the produced oxygen, which is equal to the amount of oxygen taken up by the test solution. This is printed every hour on a paper tape.

In the air space of the flask, a basket is filled with soda lime to absorb CO_2 produced in the biochemical process.

One apparatus is equipped with 6 or 12 independently operating flasks and printers. All these flasks are kept in the same room at 20 $\pm 1^{o}$ C. Magnetic stirrers, individual to each flask, allow the equilibrium between oxygen contents in the liquid and gaseous phase to be reached in a short time.

Materials

The dilution water was prepared with tap water, previously aerated by air during 24 h, and with addition of the following mineral salts: 8.5 mg/l KH_2PO_4, 21.75 mg/l K_2HPO_4, 33.4 mg/l $Na_2HPO_4 \cdot 7H_2O$, 1.7 mg/l NH_4Cl, 22.5 mg/l $MgSO_4 \cdot 7H_2O$, 27.5 mg/l $CaCl_2$, 0.25 mg/l $FeCl_3 \cdot 6H_2O$.

The nutrient medium was prepared by dilution of 200 mg yeast extract or glucose in 1 liter of the dilution water. The tested solutions were prepared by adding the determined volumes of the tested substances stock solutions into the dilution water.

As inoculum the following seeds were used:

- the municipal wastewater, obtained from the Warsaw sewerage and aerated for 24 hours before use

- the supernatant sample from the laboratory activated sludge installation

- the activated sludge from the same installation.

In the tests of activated sludge respiration in the presence of FWAs, the test solutions were prepared with settled activated sludge, to which the FWAs solutions in the dilution water were added.

Measurements

In every case, the BOD was automatically recorded every hour during the test lasting usually 1 to 3 weeks. Additionally, chemical analyses were carried out at the beginning and at the end of the tests on the concentration of the tested substance and, in some cases, the NH_3, NO_2 and NO_3 content. In some tests the concentration of the tested substance was determined every day. A flask from six flasks containing the same solution was withdrawn daily from the apparatus and its contents analyzed.

RIVER MODEL

Apparatus

The experiments were carried out in 10-liter bottles, filled with the river water and the known amounts of tested substances. The water in the bottles was stirred mechanically at a rate causing the aeration conditions similar to the aeration in the rivers. The water in the bottles was exposed to the light of the fluorescent tubes (at the intensity of approximately 1000 lux). The tests were performed in all day illumination or in regulated illumination (12 h with light and 12 h in darkness).

Materials

The river water was obtained from the Vistula river upstream of Warsaw, and next it was seasoned during at least 2 weeks in the 100 liter polyethylene barrel. During this seasoning the water was aerated by bubbling the air. The test solutions were prepared by adding the tested substances stock solutions into this river water.

Measurements

From the river models, the samples were withdrawn periodically and analysed. The tested substance concentration and in the certain tests also NH_3, NO_2 and NO_3 content were determined. The frequence of sampling and analyzing depended on the speed of biodegradation of any tested substance, usually it was made every day.

TREATABILITY TESTS

Apparatus

The test of the treatability of wastewater containing tested substances was carried out by means of a laboratory activated sludge unit, presented schematically in Fig. 1.

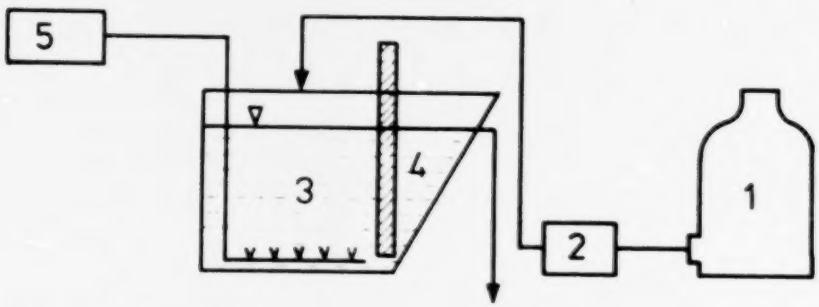


Figure 1. Laboratory activated sludge unit.

This unit consisted of a storage tank 1 (30 liter volume), containing the wastewaters with tested substance, the peristaltic pump 2 feeding the aeration chamber 3 with wastewater. The aeration chamber was separated by the wall from the settler 4. The volume of aeration chamber was equal to 1 liter, the volume of settler 0.5 liter. The mixed liquor in the chamber was aerated by means of the air pump 5.

The parameters of operation were:

- retention time: t_r= 1 to 6 hours.
- sludge age: θ = 5 days.
- MLSS: m = 3 to 4 g/l (dry mass).

Materials

The activated sludge was obtained from the municipal treatment plant and acclimatized during 2 weeks in the storage unit, similar to the unit presented above, but larger (volume of aeration chamber equal 8 liter).

The wastewaters were simulated by preparing a solution of 0.6 g/l meat extract, 0.12 g/l NH_4Cl, 0.1 g/l $MgSO_4 \cdot 7H_2O$, 0.033 g/l $NaHCO_3$ in the tap water. BOD_5 of these wastewaters was equal to ca. 360 mg/l O_2; COD was ca. 420 mg/l O_2. These wastewaters were used to feed the activated sludge units. Usually 4 units were in operation simultaneously. The test units were fed with the wastewaters, with addition of the tested substance at known concentration. The control unit, operating simultaneously was fed with wastewaters without any addition of test substances.

Measurements

The following analyses were carried out:

- the determinations of tested substance concentration in the wastewaters flowing into the test units and in the effluent,

- the determination of COD in the inflow and in the effluent,

- the MLSS (dry mass).

Additionally, the technological parameters were controlled. The retention time was controlled by means of the effluent volume measurements and the DO concentration was maintained at 2 mg/l or higher.

TOXICITY TESTS

Two kinds of bioassays were carried out. The first was the determination of the lethal concentrations of tested substances, i.e. the concentrations causing the death of 50 percent organisms used in the test during 24 or 96 hours.

LC_{50}- 24 h or LC_{50}- 96 h.

The following organisms were used in the bioassays: the fish was Lebistes reticulatus and the crustacean was Daphnia magna.

In the second type of bioassay, the influence of tested substances on the changes of the chlorophyll content in the cells of algae Chlorella was analyzed.

Tests with Lebistes reticulatus

The tests were carried out following the procedure described in Standard Methods (53).

In every test, 10 test organisms were put into the 400 ml solution of tested substance in the open vessel. The organisms dead after 24, 48, 72 and 96 hours were counted and removed to avoid the pollution of test solutions. In this kind of test, the concentration of tested substance may decrease slightly during the test.

Tests with Daphnia magna

In every test 10 organisms were put into the 200 ml solution of tested substance in an open vessel. The organisms dead after 24 hours were counted.

Tests with algae

The procedure of tests was based on a method recommended by EPA (5). The tests with use of algae Chlorella consisted in the measurement of the chlorophyll "a" content changes after 7 days of exposure to the presence of the tested substance. The tests were carried out in the 300 ml conical flask, containing tested substances in 100 ml nutrient solution, inoculated with 20 ml supernatant of algae culture. The culture of algae was kept at 18 ^{o}C ±1 ^{o}C and illuminated with the fluorescent tubes.

Materials

All test solutions were prepared with use of activated carbon treated tap water, pH equal 7.6 to 8, the calcium content 80 mg/1 and magnesium content 15 mg/1. Before use, the water was aerated.

The tested substances were always added to the test solutions, using the same stock solutions as in the biodegradability tests. The concentrations of tested substance in the test solutions represented always a geometric series, e.g. 1.0, 1.8, 3.2, 5.6, and 10 mg/1.

Test organisms

The fish Lebistes reticulatus were selected before the tests, so that all individuals were the same age (just before they revealed the sexual dimorphism).

The population of the crustacean Daphnia magna used in the tests was prepared in the following way. The matured females of Daphnia were put into the aquarium in the amount of 20 to 30 individuals. After 1 to 2 days, the new generation was hatched. After the next 5 days, these young organisms were used for the tests.

The algae Chlorella sp. wild strain were raised with use of nutrient solution prepared following the EPA standard (5). The test solutions contained the same nutrient.

SUPPLEMENTARY EXPERIMENTS

The proper interpretation of the results of experiments needs some information on the physico-chemical properties of tested substances. If this information was not available in the literature, additional experiments were carried out to obtain the needed data.

So, the following experiments were made:

- the tests of volatility of MEK, DCDEE and OCP from the dilute aqueous solutions

- tests of photolytic decomposition of FWAs

- tests of adsorption of FWAs on the flocs of activated sludge.

The tests of volatility were made by means of dry air bubbling through the dilute solutions of tested substances and measurement of the loss of the sample weight and the decrease of tested substance concentration in the sample. Henry's coefficient of equilibrium between the concentration of tested substance in the liquid and the gaseous phase was calculated.

The tests of photolysis consisted of the measurements of FWAs concentration changes in the solutions of FWAs in the distilled water,

exposed to the light of fluorescent tube at the known intensity. As pH has the influence on this process, the solutions contained buffers, so that photolysis was tested in the pH range 4 - 9.

The tests of FWAs adsorption were made by means of measurements of FWAs concentration decrease in the supernatant after the contact with activated sludge lasting 1 hour. At the same time, the activated sludge dry mass was determined which permitted the Langmuir isotherm coefficients to be calculated.

ANALYTICAL PROCEDURES

COD was determined by use of dichromate method with addition of $HgSO_4$ and $AgSO_4$. The samples were kept at the boiling temperature under the reflux condenser during 2 hours and after cooling they were titrated with the solution of Mohr's salt in the presence of 1,10-phenanthroline-ferrous sulfate complex.

The Kjeldahl nitrogen was determined by means of digestion with conc. H_2SO_4 without the former distillation of NH_3. After digestion, the ammonia was distilled off and determined by means of Nessler method. The Kjeldahl nitrogen content was calculated by substraction of NH_3 content in the raw sample from the result of the determination.

Nitrites determinations were made by spectrophotometric method with sulfanilamide and N-(1-naphtyl)-ethylenediamine.

Nitrates were reduced to nitrites by means of amalgamated cadmium and determined as NO_2 by the method described above.

Mixed liquor suspended solids MLSS (dry mass) were determined by filtering a known amount of mixed liquor through Whatman's filter paper and drying at 105 ^{o}C until constant weight.

Special procedures

The tested substances were determined by means of procedures developed in the frame of the present work. Three methods were used: GC, spectrophotometric and fluorometric methods. All GC determinations were made by use of the chromatograph Pye Unicam 104, equipped with flame ionization detector. Spectrophotometric determinations were carried out by means of Pye Unicam spectrophotometer SP 600 equipped with cells of 1 cm light path. Fluorometric determinations were made by use of the fluorometer Aminco.

Methylethyl ketone, MEK

Gas chromatographic method was used. The column, 2.1 m long and 4 mm diameter, was filled with Porapak Q (polystyrene), coated with polyethylene glycol 1000 as stationary phase. The carrier gas (nitrogen) flow rate was 30 ml/min. The temperature of column was 83 °C. The aqueous samples of MEK were injected in the amount of 5 µl each. The retention time was

9 min 45 sec. The area of the peaks was proportional to the MEK concentration within the range 0.3 to 200 mg/l.

Dimethyl amine, DMA

The GC method was used. The column 2.7 m in length and 4 mm in diameter was filled with Chromosorb T (PTFE), 30 - 60 mesh, with 20 percent addition of Triton X - 100 and 2 percent addition of KOH. Carrier gas (nitrogen) flow rate was 60 ml/min. The column temperature was 96 ^{o}C, the retention time 1 min 45 sec. The samples of DMA were treated with KOH solution to keep pH ca. 12 and were injected into the apparatus as aqueous solution in the volume of 5 µl. The detection limit was 1 mg/l DMA.

Dimethyl formamide, DMF

DMF was determined by means of hydrolysis with use of conc. HCl (1+1) and heating the sample on the boiling water bath under reflux condenser for 1 hour. After cooling, KOH was added to reach pH ca. 12 and next the DMA involved was determined by GC method.

Para-nitrophenol, PNP

The spectrophotometric method was applied. The quinone form of PNP forms with NaOH the yellow pseudo-salt intensively colored. The absorbance of the light at the wavelength 405 nm is proportional to the PNP content within the range 0.2 to 10 mg/l.

Ortho-chlorophenol, OCP

The spectrophotometric method was applied. The samples were treated with the 4-aminoantipyrine and potassium ferricyanide. The involved indophenol dye was measured spectrophotometricly at light wavelength 510 nm. Direct determination was possible within a range of OCP concentration from 0.5 to 5 mg/l. The substances contained in the yeast extract, mineral nutrients and in the river water did not interfere with the determinations.

Trichlorophenol, TCP

TCP was determined similarly as OCP with 4-aminoantipyrine method. The absorbance of the formed dye was measured at the wavelength 510 nm. Direct determination of TCP in the tested solutions is possible within a range of concentrations 0.5 to 5 mg/l.

Dichlorodiethyl ether, DCDEE

DCDEE was determined by means of GC method. The column 2.1 m long and 4 mm diameter was filled with Chromosorb T 40 - 60 mesh, coated with Triton X - 100 as stationary phase, added in the amount of 5 percent per weight. The flow of the nitrogen as the carrier gas was 30 ml/min. At a column temperature of 118 ^{o}C the retention time was 6 min 10 sec. The aqueous solutions of DCDEE were injected in a volume of 10 µl. The detection limit amounted to 0.2 mg/l DCDEE.

Fluorescent Whitening Agents, FWAs

The fluorometric method was applied. In this method, a UV beam passes through the cell with the tested solution and the light emitted by the solution at the direction rectangular to the UV beam is measured at the wavelength 450 nm.

Turbid samples were previously cleared by means of centrifuging at 12000 rpm. As the measured signal depends on pH, phosphate buffer was used to keep pH at the constant level of 7.2. The apparatus was standardized with quinine sulfate solution. The calibration curves were prepared individually for each FWA. The apparatus gave linear response within the FWA concentration range 1 to 10 mg/l. The solutions with higher FWAs content were adequately diluted before measurement.

SECTION 6

METHODS OF CALCULATIONS

RESPIROMETRIC DATA

Long Term BOD Curve Examination

If the sample, which BOD is measured, contains the nutrient medium (yeast extract or glucose and mineral salts) with addition of the specific organic compound, which biodegradability is tested, the resulting BOD curve has usually the shape schematically presented on the Fig. 2.

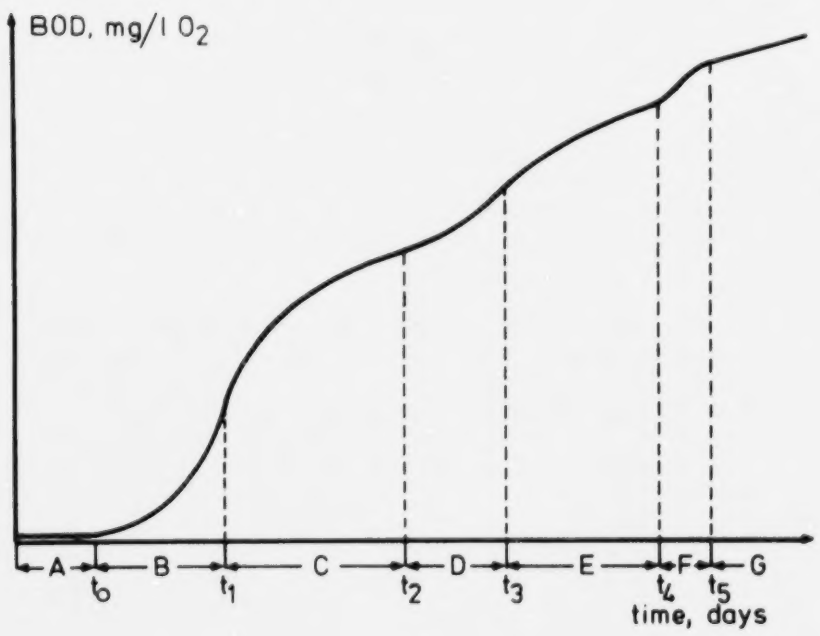


Figure 2. Typical shape of BOD curve of a solution containing nutrient medium and a specific organic compound.

The process of biochemical oxygen uptake can be divided into the following subsequent phases:

- Phase A, being lag phase, during which oxygen uptake is very small or none. This phase corresponds with the period of microorganisms acclimatization to the conditions of test.

- Phase B, being growth phase, during which the microorganisms are multiplying, so that oxygen uptake is accelerated. The curve is then concave.

- Phase C, during which the organic components of the nutrient medium are in increasing scarcity in comparison with amount of bacteria, so that the process is restrained and the BOD curve is convex.

- Phase D, being second growth phase, during which the population of bacteria, able to decompose the tested substance is growing, so that oxygen uptake for tested substance decomposition is accelerated and the BOD curve is concave.

- Phase E, analogous to phase C, during which the tested substance contents is in the increasing scarcity in comparison with the amount of bacteria able to decompose this substance. The BOD curve is then convex.

- Phase F, during which the BOD accelerates once more because of the oxygen uptake for nitrification of ammonia present in the tested solution.

- Phase G, during which the BOD curve is approximately straight line with little slope. This phase corresponds with the period, during which the nutrients and the tested substance are almost completely removed and BOD is caused mostly by endogenous respiration of microorganisms.

In almost all experiments made in the frame of the present work, the following equation can be used to describe the BOD curve sections:

- lag phase (phase A), lasting to the moment t_o:

$$y = 0, \quad 0 < t < t_o,$$

where y is BOD at the moment t

- growth phase B, lasting from the moment t_o to t_1:

$$y = c_n \cdot 10^{b_n(t-t_o)}, \quad t_o < t < t_1,$$

where c_n and b_n are constants, the last being the exponential growth coefficient corresponding with the decomposition of the nutrient medium components.

- monomolecular reaction phase C, lasting from the moment t_1 to t_2, described by the classic Streeter-Phelps equation:

$$y = y_1 + L_1 \left[1 - 10^{-k_n(t-t_1)}\right], \quad t_1 < t < t_2,$$

where y_1 is the BOD at the moment t_1, L_1 is the residual BOD at the moment t_1, and k_n is the kinetic coefficient of the decomposition of the nutrient medium components.

- the phases D and E are analogous to the phases B and C, but correspond with the tested substance biodegradation:

$$y = y_2 + C_s \cdot 10^{b_s(t-t_2)}, \quad t_2 < t < t_3,$$

$$y = y_3 + L_3[1 - 10^{-k_s(t-t_3)}], \quad t_3 < t < t_4,$$

where C_s and b_s are the constants, L_3 is the residual BOD at the moment t_3, k_s is the kinetic coefficient of tested substance biodegradation.

Nitrification phase F proceeds usually in some irregular way and was not described with any equation. In the phase G (endogenous respiration), the oxygen uptake rate is almost constant, equal Re:

$$y = y_5 + Re(t-t_5)$$

The expression $z = \log dy/dt$ transforms any of equations to the linear function of time t. Thus, the graph of the dependence of z on t has the shape schematically presented on the Fig. 3.

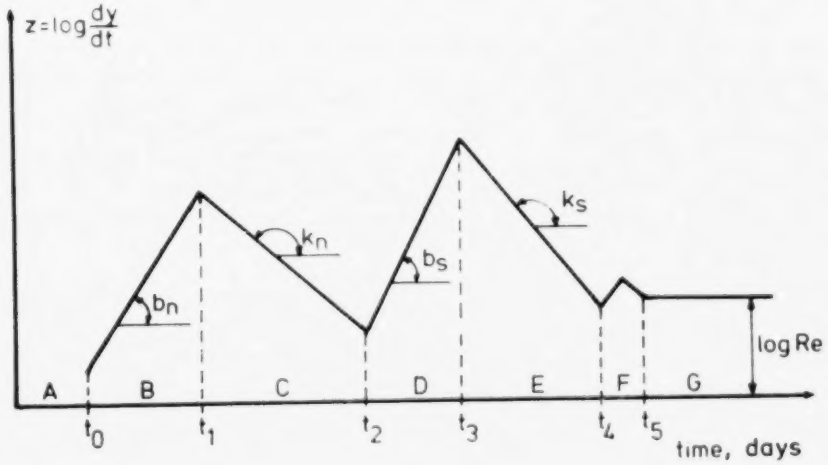


Figure 3. The typical BOD curve transformed by equation $z = \log dy/dt$.

The BOD record made every hour enables one to calculate the BOD increments Δy, approximately equal to dy/dt, the results of BOD measurements can be marked on the graph in the coordinate system: log dy/dt versus t. The slope of any straight line section drawn through the marked points indicates one of the coefficients b_n, k_n, b_s or k_s. Also the values of t_1, t_2 and t_3 can be easily read out from this graph. The final carbonaceous BOD of the tested solution can be calculated on the base of BOD recorded at the moment t_3, equal y_3 and the maximum value of log dy/dt at the moment t_3, equal z_{max}. According to the above written equations, this maximum value z_{max} is equal:

$$z_{max} = \log 2.303\ L_3\ k_s, \text{ thus: } L_3 = \frac{1}{2.303\ k_s} \cdot 10^{z_{max}}$$

The final carbonaceous BOD L_c amounts to the sum of BOD measured up to moment t_3 and BOD residual at the moment t_3, so

$$L_c = y_3 + L_3, \text{ and } L_c = y_3 + \frac{1}{2.303\ k_s} \cdot 10^{z_{max}}$$

The value y_3 is recorded and the values k_s and z_{max} are read out from the graph, so the value of L_c can be calculated.

In certain cases, when the nitrification phase F was superposed on the phase E, the recorded BOD data were corrected by substraction of theoretical oxygen demand for nitrification NOD from the recorded BOD data:

$$NOD = 3.2 \cdot \Delta\ NO_2 + 4.3\ \Delta\ NO_3, \text{ where}$$

$\Delta\ NO_2$ and $\Delta\ NO_3$ are the analytically determined increase of nitrites and nitrates contents (expressed as mg/l N).

If the final carbonaceous BOD L_c was determined in the series of samples, each containing different initial concentration c_o of tested substance, we can make the graph presenting the dependence of L_c on c_o (Fig. 4).

The slope of the straight line drawn through the marked points indicates the unit oxygen demand of tested substance L_u, i.e., the amount of oxygen needed by the solution containing 1 mg/l of the tested substance. L_u is expressed in mg O_2/mg substance. Apart from the mean value of L_u, the confidence limits 95 percent were always calculated following the Bartlett's equation. The intercept of the straight line indicates the oxygen demand caused by organic components of nutrient medium and by the inoculum used in the test.

It should be stated that in special cases, the shape of BOD curve differs from the above given pattern. For example, if the tested substance is as easily biodegradable as organic components of the nutrient medium are, the phases D and E are superposed on the phase B and C, i.e., the decomposition of the tested substance proceeds simultaneously with the decomposition of nutrient medium components. If the tested substance is not biodegradable, the phases D and E do not occur at all.

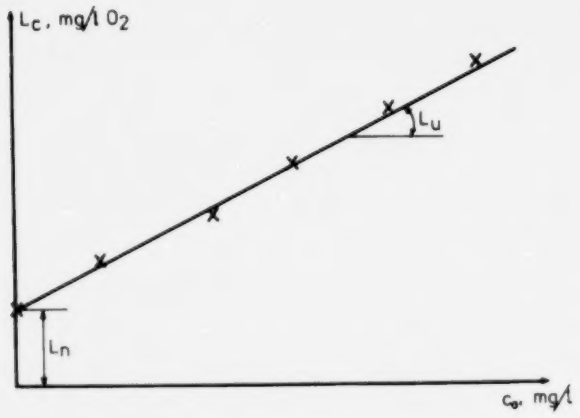


Figure 4. The dependence of final carbonaceous BOD L_c on the initial concentration of the tested substance c_o.

The Tested Substance Decomposition

In some tests, the tested substance contents were analyzed every day in subsequent samples, initially identical. (BOD was also recorded.)

On the graph z = log dy/dt versus the time the supplementary values of log c were added (where c is the tested substance concentration at the moment t). It allows to confirm that BOD process during the phase D and E is corresponding with the decrease of the tested substance contents. The slope of the straight line log c = f(t) is also the kinetic coefficient of tested substance decomposition k_s.

In the cases when ammonia was one of the final products of tested substance decomposition, the values of log $\frac{N_f}{N_f - N}$ were marked versus the time (where N_f means final NH_3 contents and N means NH_3 contents at the moment t). The slope of this line also indicates the kinetic coefficient k_s.

The Choice of Parameters Characterizing the Biodegradability

Practice shows that many of the above described parameters are not useful to characterize the biodegradability of tested substances. The reason is that their values depend on the properties of the inoculum used in the tests, and the control of these properties is very difficult. Even if the source of the seed is always the same (e.g., the wastewaters sampled from the same municipal sewage system), the quantity and kind of sewage microorganisms

highly differs in different samples. As the results of experiment should describe the properties of tested substance and not the random conditions of the test performing, it was necessary to choose those parameters which are reproducible.

It was found, that the best reproducibility can be obtained when the relative parameters were chosen, namely, the differences or the ratios of the parameters corresponding with the tested substance and the standard nutrient medium biodegradation.

Thus, the influence of a given substance on the microbiological activity can be determined by the calculation of the inhibition period of the nutrient medium biodegradation. If $t_1(0)$ and $t_1(c_o)$ denote the moments of maximum rate of nutrient medium decomposition, respectively in the blank sample not containing the tested substance and in the sample containing initially the tested substance at the concentration c_o, the inhibition time Δt_1 is equal to:

$$\Delta t_1 = t_1(c_o) - t_1(0)$$

The inhibiting influence of various concentrations of the tested substance on the yeast extract or glucose decomposition can be described by the graph $\Delta t_1 = f(c_o)$. The examination of this graph allows one to conclude whether a given substance inhibits the microbiological activity and if so, at what concentration it does.

The biodegradability of the tested substance can be determined by comparison with the biodegradability of nutrient medium components. The first method is to calculate the inhibition time of tested substance decomposition Δt_2. It is equal to:

$$\Delta t_2 = t_3(c_o) - t_1(0)$$

where $t_3(c_o)$ is the moment of maximum rate of the tested substance decomposition in the sample containing the initial concentration c_o, and $t_1(0)$ is, as formerly, the moment of maximum decomposition rate of the yeast extract or glucose in the blank sample.

The data obtained in experiments with various initial concentrations c_o, allow to prepare the graph $\Delta t_2 = f(c_o)$. Examination of this graph leads to the conclusion how much the given substance is more difficult to biodegrade than the standard substance.

Another method is to calculate the ratio r of the values k corresponding respectively with the tested substance decomposition and the decomposition of the nutrient medium components:

$$r = \frac{k_s(c_o)}{k_n(0)} ,$$

where $k_s(c_o)$ is the value of k coefficient read out from BOD graph, corresponding with the phase E of the process in the sample containing initial concentration c_o. The k_n is the k value read out from the BOD curve, phase C, concerning the blank sample ($c_o = 0$).

As formerly, the dependence of r value on c_o value can be presented graphically. If r = 1, we can conclude that the tested substance is decomposed at the same rate as organic components of the nutrient medium (after certain period of microorganisms acclimatization). If r < 1, the biodegradation of tested substance is slower, and if r > 1, it is faster than the biodegradation of the nutrient medium components.

BIODEGRADABILITY IN THE RIVER WATER

The only information gathered during the experiments with the river model was the results of determination of the tested substance concentration during the tests. In several cases this information was supplemented by the data of NH_3, NO_2 and NO_3 contents during the tests.

It was found that the process of specific organic compound decomposition in the river water can be divided into three phases. In the first phase, the decomposition is slow or does not occur. This lag phase corresponds with the period of acclimatization of microorganisms to the presence of the given substance. In the second phase, the decomposition of the tested substance proceeds at the approximately constant rate:

$$v = -dc/dt = const.$$

In the third phase, when the concentration c of the tested substance drops down below certain critical value, the decomposition proceeds at a decreasing rate and the concentration c decreases asymptotically to zero. The process in this phase usually could not be exactly observed, because the concentrations were close to the detection limit.

The above given pattern of process is illustrated by the typical graph of a tested substance decrease in the river water as shown in Fig. 5.

As the parameters characterizing the biodegradation of tested substances in the river water, the following ones were chosen:

- the time of lag phase t_o
- the decomposition rate v during the constant rate phase.

The values of the above given parameters can depend on the kind of microorganisms living in the river water used in the tests. However, it can be assumed, that they are adequate to comparatively study the fate of all tested substances in the river water.

TREATABILITY DATA

In the treatability tests, the inflowing wastewaters and the effluent from the activated sludge unit were analyzed. The determined parameters were: COD and tested substance content. Additionally, the MLSS (dry mass), the retention time and D.O. content were controlled.

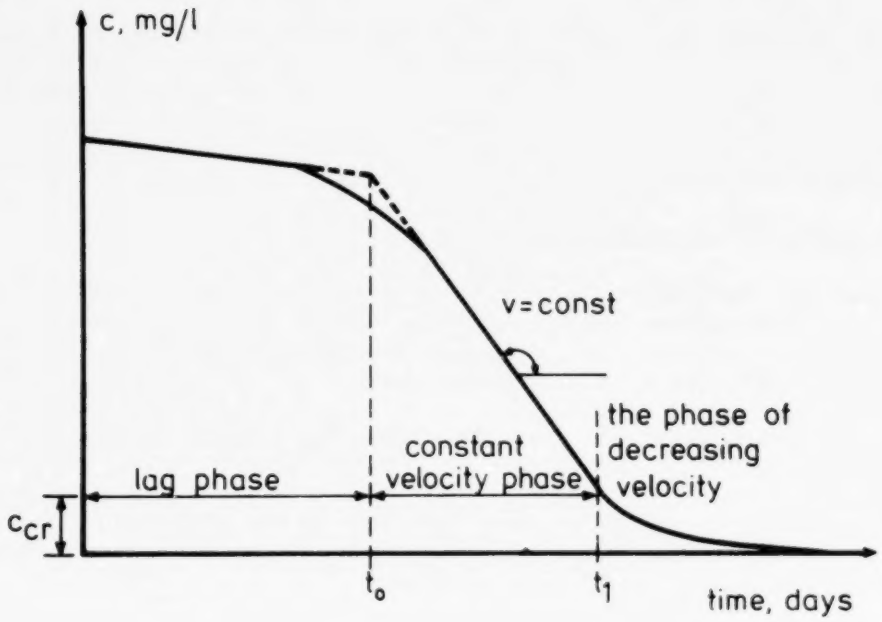


Figure 5. The typical curve of the decomposition of a specific organic compound in river water.

The simple method of treatment efficiency characterization is the calculation of coefficient K, defined as below:

$$K = \frac{c_o - c_e}{c_e} \cdot \frac{1}{mt_r}$$

where:
- c_o and c_e denote the concentration of the pollutants in the inflowing wastewaters and the effluent, respectively, mg/l
- m denotes MLSS in the aeration chamber, g/l
- t_r is the retention time, hours

The treatment efficiency coefficient K determines the treatability of a given substance by means of activated sludge, and its value is approximately independent from the technological parameters of treatment process (MLSS and retention time).

In the present work two values of K coefficient were calculated: the value K_s, corresponding with the tested substance removal, and the value K_{COD} characterizing the overall treatability of wastewaters. In this last case, the COD values of the inflow and of the effluent have to be substituted into the above equation for c_o and c_e.

Usually, when c_o was increased, the values K_{COD} and K_s decreased, but after certain time t_1, K_{COD} reached a stabilized level. Similarly, the value K_s reached the constant level after the time t_2. The values t_1 and t_2 determine the time of sludge acclimatization which restores the normal activity of microorganisms at the time t_1 and makes the microorganisms able to decompose the tested substance after the moment t_2.

As characteristics of the treatment process, the following parameters were presented:

- times of acclimatization t_1 and t_2
- overall treatability coefficient K_{COD}
- the specific substance treatability coefficient K_s.

All these characteristics are the functions of tested substance content in the inflow c_o.

TOXICITY TESTS

The 50 percent lethal concentrations LC_{50}-96 h or 24 h were calculated by use of the "probit" method. This method of calculation is based on the assumption that the mortality of the test organisms is a Gaussian function of the logarithm of the toxicant concentration. So, the observed mortality were marked on the probability paper versus the logarithm of tested substance concentration and the best fitting straight line was drawn. LC_{50} value was read out as the abscissa, corresponding with the value of ordinate equal to 50 per cent. To present the results of mortality determinations, the empirical points were marked in the coordinate system: mortality (%) versus the tested substance concentration, and next the best fitting log normal distribution curve was drawn.

SUPPLEMENTARY TESTS

The Tests of Volatility

According to the Henry's law, the concentration of a given substance in gaseous phase c_g is proportional to the concentration of this substance in the liquid phase c_l:

$$c_g = a \cdot c_l$$

If a volume dV_a of the air is bubbled through a volume V_w of solution, the decrease of volatile substance concentration in the liquid is:

$$dc_l = - c_g dV_a/V_w, \text{ or: } dc_l/c_l = -a\ dV_a/V_w.$$

At the same time, the bubbling of the air causes the evaporation of dV_w, the volume dV_w of water, equal to:

$$dV_w = \frac{0.018 \times 20}{760 \times 24} \cdot dV_a, \text{ where}$$

- 0.018 is the molecular mass of water kg/mole
- 24 is the molar volume of water vapor at the test temperature 22 °C and at the normal pressure 760 mm Hg

- 20 is the partial water vapor pressure at the test temperature mm Hg
- 760 is the atmospheric pressure, mm Hg

The rearranging of these equations leads to:

$$\frac{dc_1}{c_1} = 50.7 \times 10^3 \times a \times \frac{dV_w}{V_w} \quad \text{or} \quad \log \frac{c_1}{c_o} = 50.7 \times a \times 10^3 \log \frac{V_w}{V_o} ,$$

where c_o and V_o are the volatile substance concentration and the liquid volume at the beginning of the tests.

The results of the determinations of c_1 and the weighing of the solution giving V_w values are marked on the graph $\log c_1$ versus $\log V_w$. The slope of the obtained straight line equals the factor $50.7 \times 10^3 \times a$, so the value a can be calculated.

The blowing off rate of the volatile, but not biodegradable substance, from the aeration chamber can be calculated with use of mass balance equation:

$$c_o \cdot V_w = c_e \cdot V_w + V_a \cdot c_g , \quad \text{where}$$

- V_w and V_a are the volume rate of inflowing wastewaters and the air, l/h.
- c_o, c_e and c_g are the volatile substance concentration, respectively in the inflow, in the effluent and in the air flowing off the chamber.

As $c_g = a \cdot c_e$, the volatile substance concentration in the effluent is equal:

$$c_e = \frac{c_o}{1 + a \cdot \frac{V_a}{V_w}}$$

SECTION 7

RESULTS OF BIODEGRADATION

METHYLETHYL KETONE, MEK

Respirometric Measurements

Three series of experiments were made. In the first series, BOD was measured by means of Sapromat. The samples contained the nutrient medium (yeast extract) with various MEK additions within the range 0 - 800 mg/l. The test solutions were inoculated with municipal wastewater. In the second series, both BOD and MEK content changes were observed in the samples with initial MEK concentration equal 200 mg/l. After the second test was completed, the third series was carried out with the same conditions except that the solution remaining from the second test was used as inoculum (test with the microorganisms acclimatized to the presence of MEK).

In the first series, in which different initial concentrations of MEK c_o were applied, the phase of nutrient medium decomposition was superposed on the phase of MEK decomposition. In Figs. 6, 7 and 8 the following relations are presented:

- final carbonaceous BOD (L_c as function of initial MEK content c_o)
- the time of process inhibition Δt_1 as a function of c_o
- the ratio r of the coefficient of MEK decomposition k_s to the coefficient of nutrient medium components k_n.

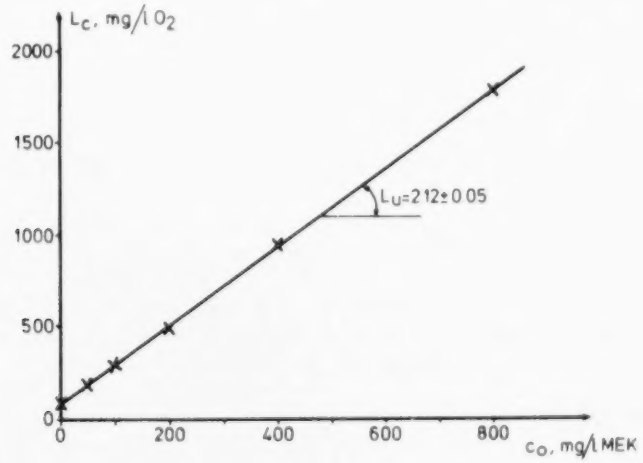


Figure 6. The final carbonaceous BOD L_c as function of initial MEK content c_o.

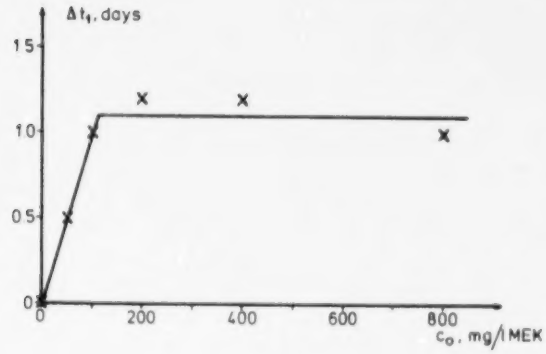


Figure 7. Lag phase duration Δt_1 as function of c_o.

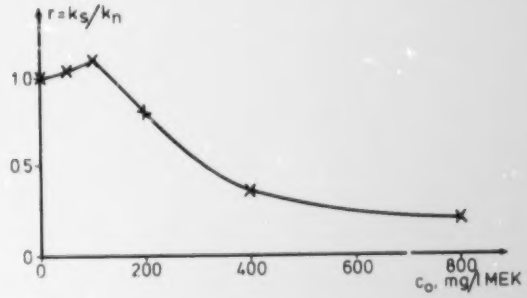


Figure 8. Dependence of the ratio r on initial MEK concentration c_o.

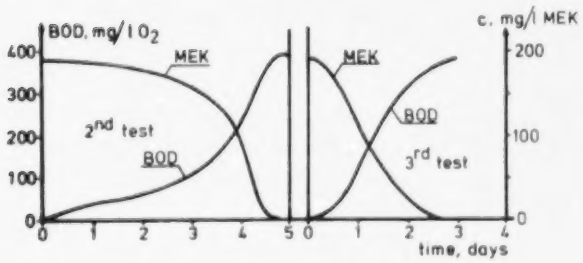


Figure 9. Decrease of BOD and MEK during the respirometric test.

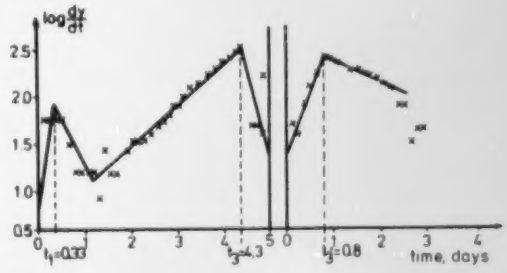


Figure 10. Linearized BOD curve from Figure 9.

The results of the second and third series are presented graphically in Figs. 9 and 10.

Discussion

It may be assumed that the basic reaction of MEK biodegradation follows the equation:

$$CH_3.CO.C_2H_5 + \frac{11}{2} O_2 \longrightarrow 4\ CO_2 + 4\ H_2O$$

The stoichiometric value of oxygen uptake in the above given process of MEK oxidation amounts to 2.44 mg O_2/mg MEK. The experimental value (Fig. 6) was determined as equal to 2.12 ±0.05 mg O_2/mg MEK.

Comparing of experimental and stoichiometric values leads to the conclusion that ca. 87% of MEK is mineralized and the resting 13% is assimilated by microorganisms to produce the biomass.

Determination of inhibition time of biochemical processes in the presence of MEK leads to the conclusion that MEK at a concentration of 100 mg/l and above causes the inhibition of the microbiological processes during 1 day in comparison with the process in the blank sample.

The coefficient k_s of monomolecular reaction of MEK decomposition decreased at the initial MEK concentrations of 200 mg/l and above. The reason may be that at higher MEK concentrations the mineral nutrients may not be adequate, thus slowing the process.

The acclimatization testing showed that the previous adaptation of the seed used to inoculate the sample significantly shortens the lag phase.

Biodegradation in the river water

The results of MEK biodegradation in the river water are presented on Fig. 11. The right side of the graph presents the MEK content changes after the repeated addition of MEK to the water in which the first portion of MEK was decomposed (i.e. to the water containing acclimatized microorganisms).

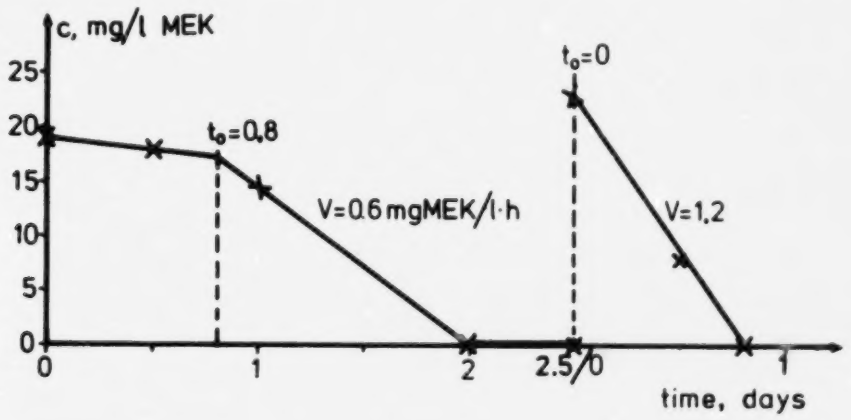


Figure 11. The decomposition of MEK in river water.

It is visible that MEK is easily biodegraded by the microorganisms living in the river water, especially when they are previously acclimatized to MEK.

Treatability tests

During the experiments two activated sludge test units and one control unit were operating in parallel The first was fed with wastewater containing 200 mg/l MEK; the second one 400 mg/l MEK. The results of MEK content in the effluent and the results of calculations of the overall treatability coefficient K_{COD} are presented in Figs. 12 and 13.

It is visible that MEK is easily removed from the wastewater after acclimatization of the activated sludge, lasting 8 to 9 days. The waste-

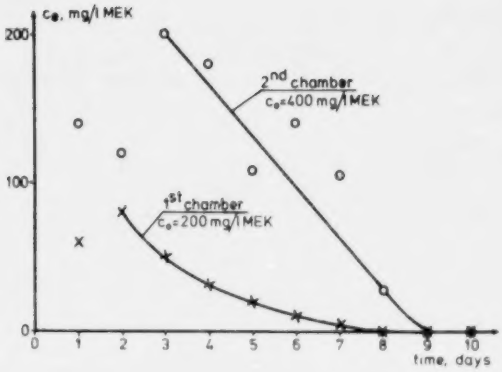


Figure 12. MEK removal by activated sludge.

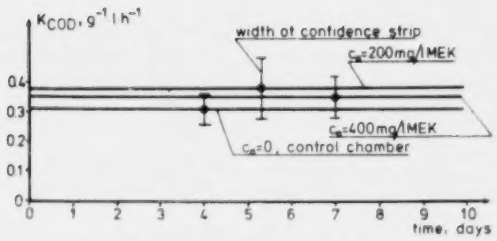


Figure 13. The treatability coefficient K_{COD} of wastewater containing MEK.

water treatment (COD removal) is practically independent from MEK content in the inflow. The differences between K_{COD} values in the control unit and in the test units were statistically not significant.

DIMETHYL AMINE, DMA

Respirometric measurements

Seven series of measurements were made. In the first three series different initial DMA concentrations were applied, whereas in the remaining series, the samples contained initially the same DMA concentrations.

TABLE 6. THE CONDITIONS OF RESPIROMETRIC MEASUREMENTS OF DMA BIODEGRADABILITY

No.	Number of samples in the series	Test duration, days	Nutrient medium	Seed	Range of concentration, mg/l
I	5	18	yeast extr.	sewage	0 - 135
II	6	12	yeast extr.	sludge	0 - 270
III	8	13	glucose	sludge	0 - 900
IVa	4	5	"	sludge	180
IVb	4	5	"	adapted sludge	180
V	9	11	"	sludge	170
VI	6	7	"	sludge	185
VII	5	8	"	sludge	180

The exemplary curves of BOD, DMA decrease and NH_3 increase are shown in Fig. 14 (the data resulting from series V). On Fig. 15 the same data are presented in the linearizing coordinates system.

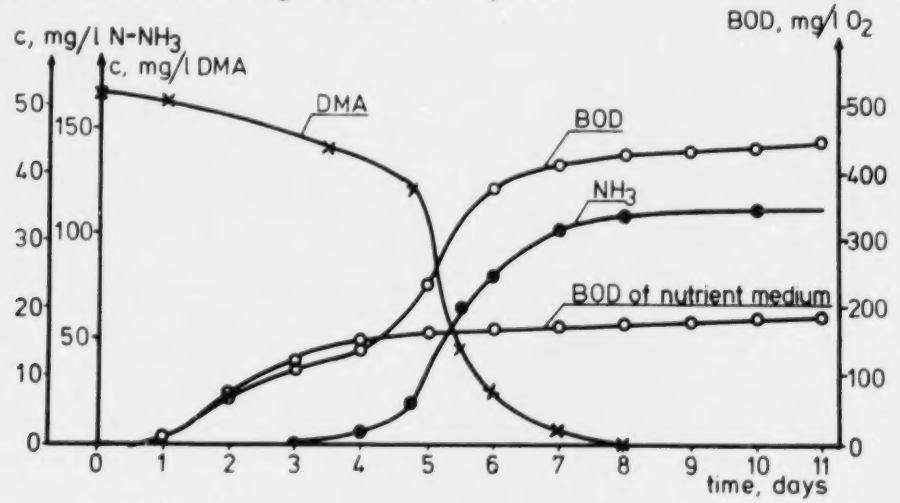


Figure 14. BOD curve, DMA decrease and NH_3 increase during the respirometric test.

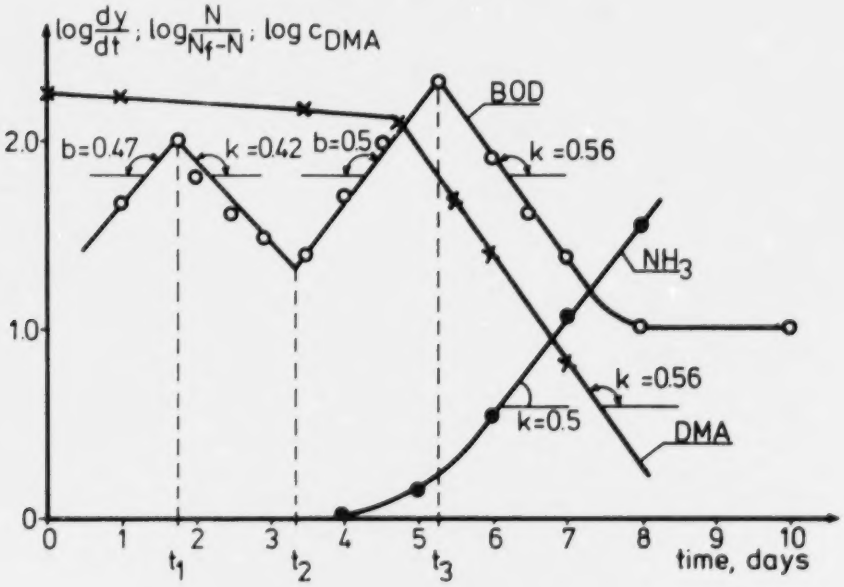


Figure 15. The curves from Figure 14 after linearizing transformation.

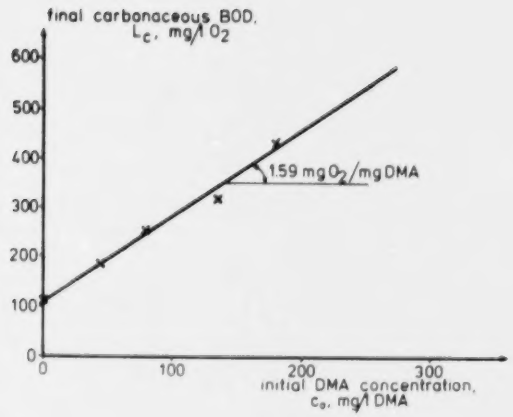


Figure 16. The final carbonaceous BOD L_c as function of initial DMA content c_o.

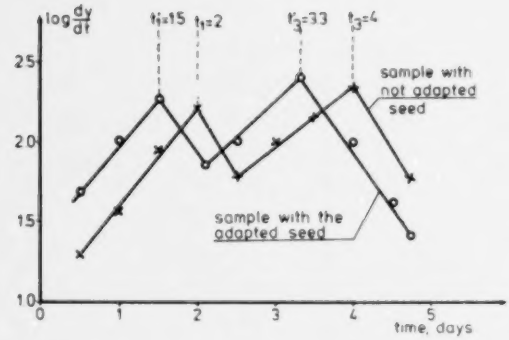


Figure 17. The influence of the seed acclimatization on the DMA biodegradation process.

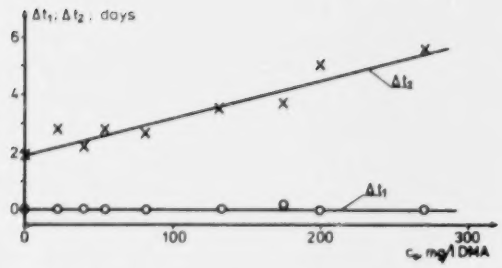


Figure 18. The inhibition time of glucose decomposition Δt_1 and of DMA decomposition Δt_2 as function of the DMA initial content c_o.

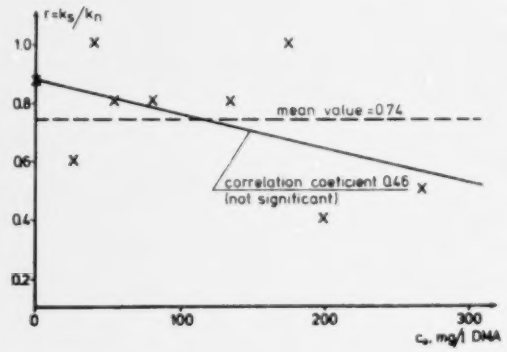


Figure 19. The dependence of the ratio r on the initial DMA concentration c_o.

The dependence of final carbonaceous BOD L_c on the initial DMA concentration c_o is exemplified in Fig. 16 (data obtained in the II series). The mean value of L_c calculated from data obtained in all 7 series was equal to 1.56 $\pm$0.05 mg O_2/mg DMA. The ammonia produced in the biodegradation of DMA N_u calculated in a similar way was N_u = 0.21 $\pm$ 0.006 mg NH_3-N/mg DMA. Fig. 17 presents the results of biodegradation tests in which the samples were seeded with non-acclimatized and acclimatized inoculum to DMA.

The data obtained in all 7 series were marked on the graphs in a similar way, and the moments of maximum BOD t_1 and t_2 were read out. Next, the time of inhibition of the nutrient medium decomposition Δt_1 was calculated and presented in Fig. 18 as the function of initial DMA contents c_o. In the same Fig. 18 the inhibition time of DMA decomposition Δt_2 is presented also.

Additional analyses of final DMA contents have shown that at initial concentrations up to 270 mg/l DMA was completely decomposed; whereas, DMA decomposition at initial concentrations of 450 and 900 mg/l proceeded more slowly.

The velocity of DMA decomposition in comparison with the velocity of yeast extract or glucose decomposition is characterized by the values of r, defined as $r = k_s/k_n$, where k_s is the coefficient of DMA decomposition, k_n is the coefficient of decomposition of glucose or yeast extract. The dependence of r on initial DMA concentration c_o is shown in Fig. 19.

Discussion

According to the equation of DMA oxidation: $(CH_3)_2NH + 3O_2 \longrightarrow 2CO_2 + 2H_2O + NH_3$, the unit oxygen uptake should amount to 2.13 mg O_2/mg DMA, and the unit ammonia involved should be N_u = 0.31 mg N/mg DMA. Comparison of this last value with experimentally determined N_u = 0.21 leads to the conclusion that approximately 2/3 of DMA content is mineralized according to the above equation and 1/3 is assimilated by the microorganisms.

The experimentally determined value of unit oxygen demand L_u is equal to 1.56 in comparison with the stoichiometric value of 2.13 mg O_2/mg DMA. Therefore, the most probable summary reaction equation is as follows:

1 mg DMA
- 2/3 mg DMA $\xrightarrow{1.42 \text{ mg } O_2}$ 0.25 mg NH_3 + 1.3 mg CO_2 + 0.53 mg H_2O + energy
- 1/3 mg DMA $\xrightarrow[+ \text{ nutrients}]{0.14 \text{ mg } O_2}$ biomass + byproducts

The ratio of assimilated nitrogen coming from DMA to the final BOD is equal to 1 mg NH_3-N:15 mg O_2 which is near to the value of N:BOD ratio optimal in the treatment process.

The results of BOD determination show that DMA at concentrations up to 270 mg/l do not inhibit the microbiological activity which is proved by the horizontal course of graph $\Delta t_1 = f(c_o)$ (Fig. 18). The DMA decomposition proceeds with some delay which increases as the initial DMA content increases. Additional analyses of final DMA content show that at c_o = 450 and c_o = 900 the DMA decomposition was strongly inhibited during all 13 days of testing.

The ratio of DMA decomposition coefficient to the coefficient corresponding to the nutrient medium decomposition is ca. 0.75 and it depends just a little on the DMA contents within the range of c_o 0 - 270 mg/l (the dependence is statistically not significant). Therefore, it can be stated that DMA biodegradation after a certain time of microorganisms acclimatization proceeds with a velocity not much lower than the velocity of nutrient medium decomposition.

The acclimatization of the inoculum to the DMA shortens the inhibition periods ca. 0.5 day in comparison with the biodegradation process without acclimatization. Beyond this, the process is very similar in both cases.

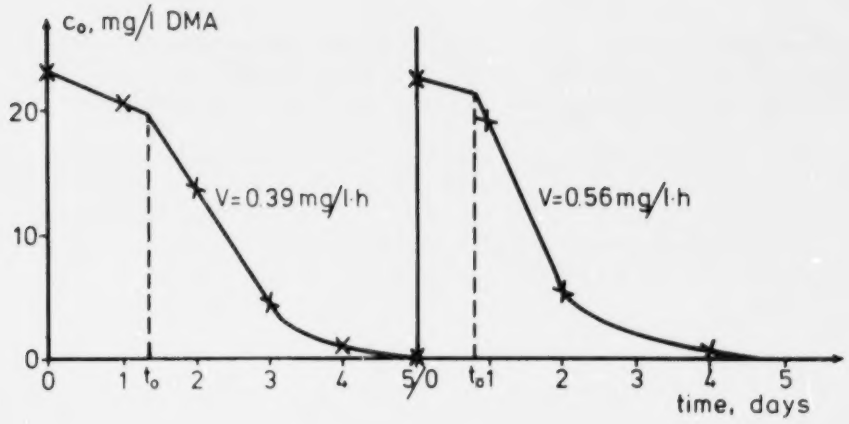


Figure 20. The decomposition of DMA in river water.

TABLE 7. KINETIC PARAMETERS OF DMA BIODEGRADATION IN RIVER WATER

	Lag phase t_o, days	Decomposition velocity (v), mg/l·h
Without acclimatization	1.3	~ 0.4
After acclimatization	0.8	~ 0.55

Biodegradation in the river water

During the test, the DMA content was measured. When DMA content had decreased down to zero, the next dose of DMA was added once more to the same solution (adaptation test). The results are presented in Fig. 20. The kinetic parameters read out from the graph are gathered in Table 7. The data show that DMA can be biodegraded in the river water during several days. The process is advanced and accelerated by previous acclimatization of microorganisms to the DMA.

Treatability tests

Three series of experiments were made. In the first series the activated sludge unit was fed with wastewater containing 20 mg/l DMA. In the second series two activated sludge units were operating in parallel (DMA content in the inflow was 45 and 90 mg/l during the first week and changed to 90 and 135 mg/l, respectively). In the third series one unit was fed with wastewater with 90 mg/l DMA; the second one with 135 mg/l DMA.

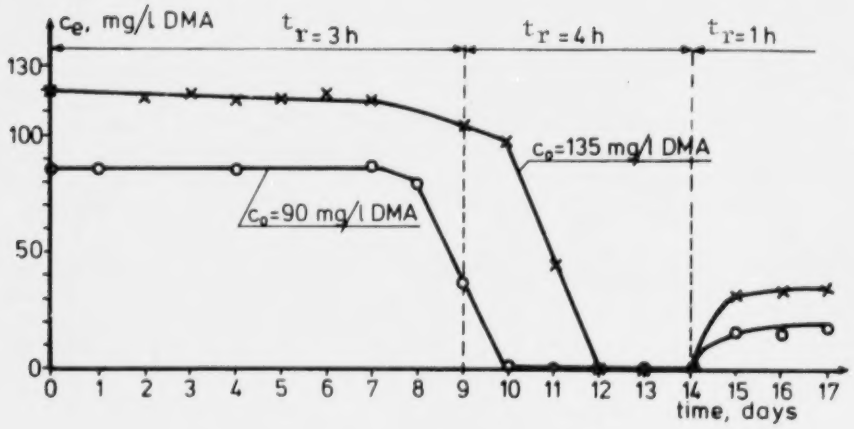


Figure 21. DMA removal by activated sludge.

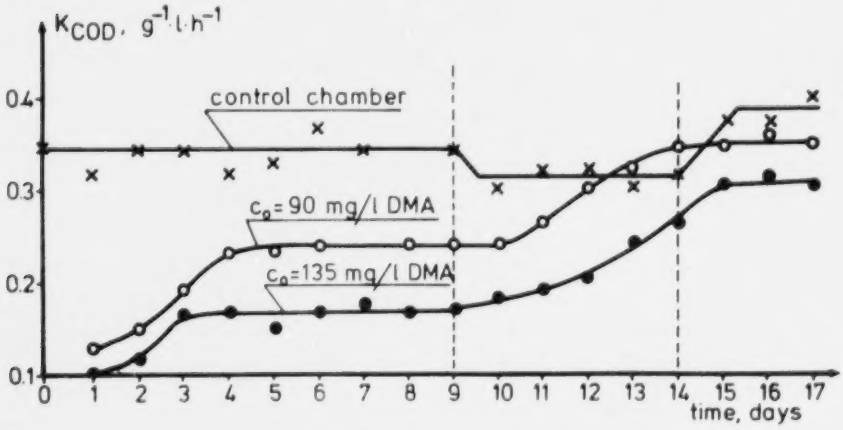


Figure 22. The treatability coefficient K_{COD} of wastewater containing DMA.

The results of DMA removal determinations and the calculations of the overall treatability coefficient K_{COD} are presented in Figs. 21 and 22 (third series).

On the basis of these graphs, as well as of the data obtained in the remaining series, the following conclusions can be made.

- The overall treatability, characterized by the value of K_{COD}, did not depend on the DMA content (within the period of K_{COD} value stabilization after acclimatization of the sludge). The differences of these K_{COD} values corresponding with the control unit and two test units were statistically not significant.

- The DMA treatability after several days of sludge acclimatization was high and no DMA could be detected in the effluent at DMA content in the inflow c_o up to 135 mg/l and with a retention time of 4 hours. However at the very

short retention time (1 hour) and at c_o equal to 90 and 135 mg/l, the effluent contained measurable quantities of DMA which enabled one to calculate K_{DMA} value equal to 2.0 $g^{-1} \cdot l \cdot h^{-1}$.

- The acclimatization of the activated sludge is characterized by the values t_1 of the time necessary to stabilize the COD removal rate after any increase of DMA content in the inflow and by the value t_2 required for DMA removal rate stabilization. These values are gathered in Table 8.

TABLE 8. THE COURSE OF ACTIVATED SLUDGE ACCLIMATIZATION TO DMA

Series	DMA in inflow c_o, mg/l	Time t_1, days	Time t_2, days
I	20	-	6
IIa	45	5	5
after c_o increase up to ...	90	3	0
IIb	90	5	6
after c_o increase up to ...	135	4	0
IIIa	90	15	10
IIIb	135	14	12

TABLE 9. THE CONDITIONS OF THE RESPIROMETRIC MEASUREMENTS OF DMF BIODEGRADATION

No.	Number of samples in the series	Test duration, days	Nutrient medium	Seed	Range of concentration, mg/l
I	4	10	yeast extr.	sludge	0 - 440
II	4	9	glucose	sludge	0 - 440
III	6	7	"	sludge	145
IV	6	9	"	sludge	145
V	6	8	"	adapted sludge	145
VI	6	9	"	sludge	290
VII	6	6	"	adapted sludge	290

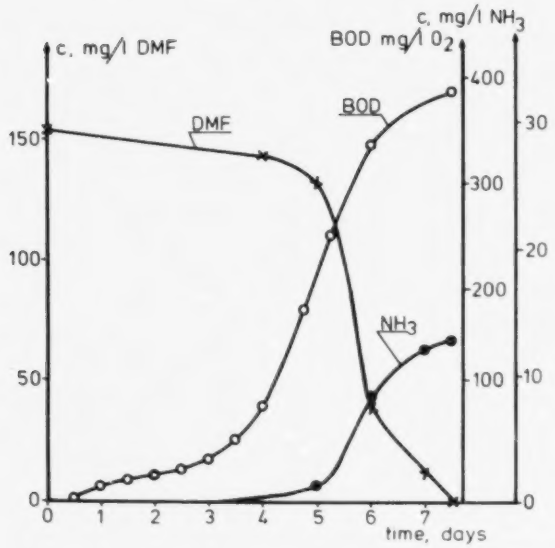


Figure 23. BOD curve, DMF decrease and NH_3 increase during the respirometric test.

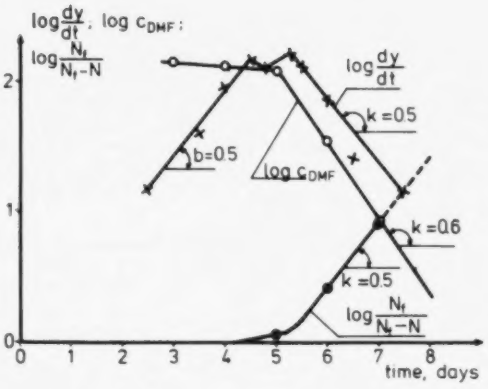


Figure 24. The curve from Figure 23 after linearizing transformation.

DIMETHYL FORMAMIDE, DMF

Respirometric measurements

Seven series of tests were carried out. In two series, BOD of the samples with various DMF content was measured. In the other series, just one DMF concentration was used but the measurements consisted of BOD, DMF, NH_3, NO_2 and NO_3 determinations. The conditions of the tests are given in Table 9.

The exemplary curves of BOD, DMF decrease and NH_3 increase are given in Fig. 23 (the data coming from the third series). The same data after the linearizing transformation are presented in Fig. 24.

On the basis of the data obtained in all the series, the final carbonaceous BOD L_c was calculated. The exemplary graph presenting L_c as a function of initial DMF concentration c_o is given in Fig. 25 where the final ammonia content is also marked.

The mean value of unit oxygen demand related to the decomposition of 1 mg DMF is equal to: $L_u = 1.40 \pm 0.02$ mg O_2/mg DMF. The amount of ammonia produced in the process of the decomposition of 1 mg DMF is equal to: $N_u = 0.087 \pm 0.004$ mg NH_3-N/mg DMF.

The influence of the previous acclimatization of the seed microorganisms on the DMF decomposition kinetics is visible from Fig. 26 where the linearized BOD curves are marked: curve I corresponds with the sample seeded without acclimated inoculum and curve II corresponds with the sample

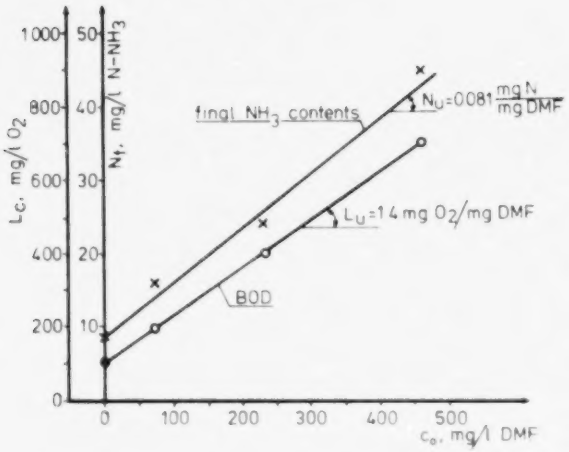


Figure 25. The final carbonaceous BOD L_c and the final ammonia content as a function of initial DMF content c_o.

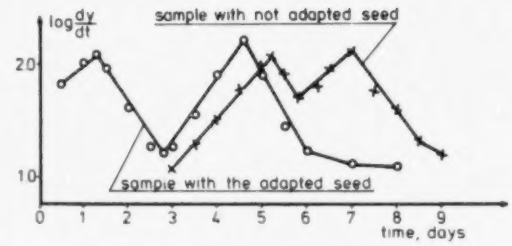


Figure 26. The influence of the seed acclimatization on the DMF biodegradation process.

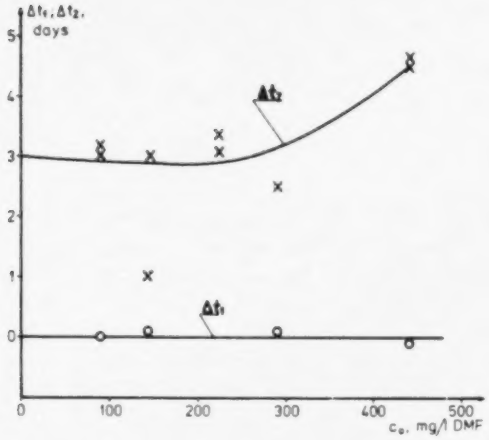


Figure 27. The inhibition time of glucose decomposition Δt_1 and of DMF decomposition Δt_2 as a function of the DMF initial content c_o.

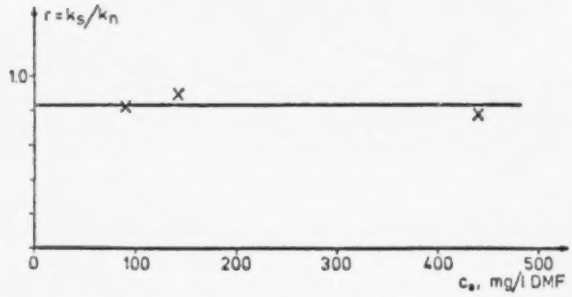


Figure 28. The dependence of the ratio r on the initial DMF concentration c_o.

seeded with solution remaining from the former sample.

The DMF decomposition kinetics are characterized by the curves presented in Figs. 27 and 28. In Fig. 27 two curves are presented, both curves being calculated on the basis of data coming from all 7 series, and representing the following:

- the dependence of inhibition time Δt_1 of nutrient medium decomposition on the initial DMF concentration c_o.

- the dependence of inhibition time Δt_2 of DMF decomposition on c_o.

In Fig. 28 the ratio r of the coefficient k_s of DMF decomposition to the coefficient k_n of nutrient medium decomposition in the blank sample is plotted versus c_o.

Discussion

Assuming that the basic reaction of biochemical oxidation of DMF follows the summarizing equation:

$$H.CO.N(CH_3)_2 + 7/2\ O_2 \longrightarrow 3\ CO_2 + 2\ H_2O + NH_3$$

we can calculate the stoichiometric oxygen uptake as equal to 1.53 mg O_2/mg DMF, and the unit ammonia production equal to 0.19 mg NH_3-N/mg DMF.

The measured unit oxygen uptake was equal to 1.40 mg O_2/mg DMF which corresponds to 90 percent of the stoichiometric value. The unit ammonia production was measured as equal ca. 0.09 mg NH_3-N/mg DMF, i. e. a little less than 50 percent of stoichiometric value. This last figure shows that the ratio of nitrogen assimilated by microorganisms to the carbonaceous BOD is equal to ca. 1:14 (mg N:mg O_2) which is near to the optimal ratio in the activated sludge treatment.

The determinations of the kinetic parameters lead to the conclusion that DMF at concentrations up to 440 mg/l do not affect the nutrient medium biodegradation which is proved by the horizontal course of straight line $\Delta t_1 \cong 0$ (Fig. 27).

The inhibition time of DMF decomposition Δt_2 changed irregularly. Up to DMF concentration 300 mg/l this inhibition time was equal to ca. 3 days and at the concentration of 440 mg/l this time increased up to 4.5 days. At all concentrations tested, the DMF decomposition was always complete, i.e. no final DMF content was measurable.

The previous acclimatization of the seed microorganisms to the DMF presence shortens the inhibition time of nutrient medium biodegradation (t_1 is average 3.5 days shorter) as well as DMF biodegradation (t_3 is average 3 days shorter).

The monomolecular reaction rate coefficient k_s of DMF decomposition amounts to 80 percent of k_n value corresponding with yeast extract or glucose biodegradation. Therefore, it can be stated that except for a period of delay needed for microorganisms acclimatization, the biodegradation of DMF processes is not much slower than the decomposition of above mentioned standard substances.

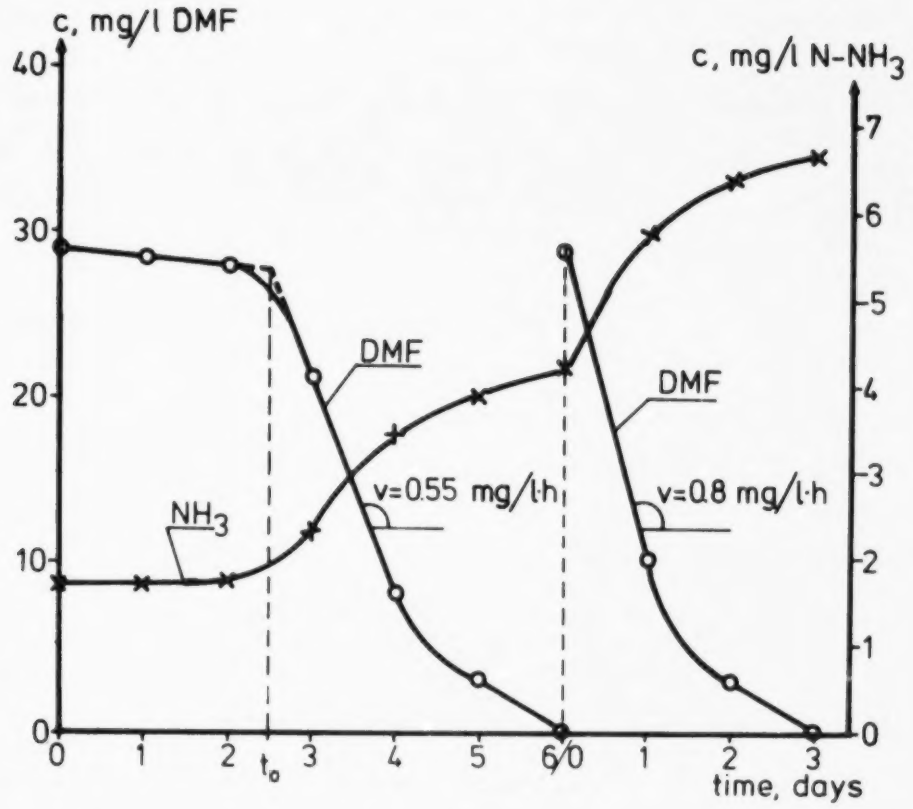


Figure 29. The decomposition of DMF in river water.

Biodegradability in the river water

Three series of measurements were made. In the first and second series, the DMF was added to the fresh river water. The initial DMF content was equal to ca. 30 mg/l. The third series consisted of the repeated addition of DMF to the water in which DMF was completely decomposed during the second series, i.e. to water with acclimatized microorganisms. The results of the second and third series are presented in Fig. 29.

The tests without acclimatized microorganisms gave the following results (mean of the values from first and second tests):

- lag phase t_o = 2 days
- the degradation rate v = 0.5 mg/l·h

The test with previously acclimatized microorganisms showed the following results:

- lag phase $t_o \cong 0$

- the degradation rate v = 0.8 mg/l·h

These values prove that DMF is easily biodegraded in the river water, but it needs a certain period of microorganisms acclimatization.

The treatability tests

One test lasting 42 days was made in which wastewater containing 70 mg/l DMF was treated. The results of DMF determinations in the effluent and the values of overall treatability coefficient K_{COD} are given graphically in Figs. 30 and 31.

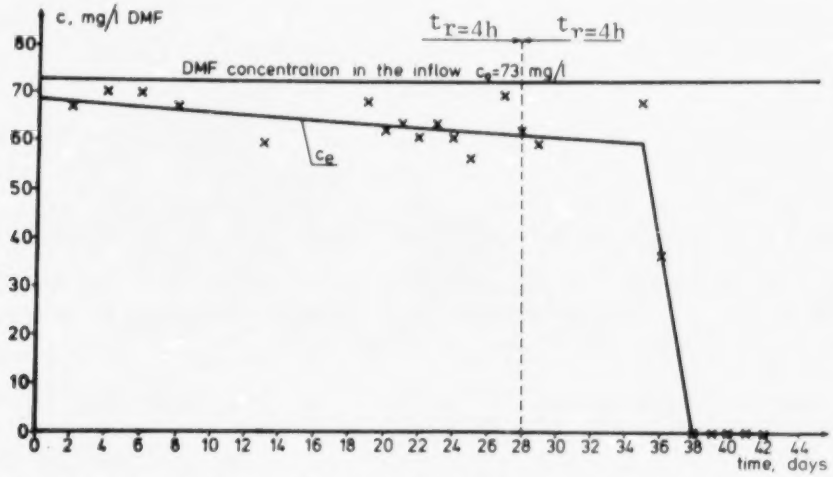


Figure 30. DMF removal by activated sludge.

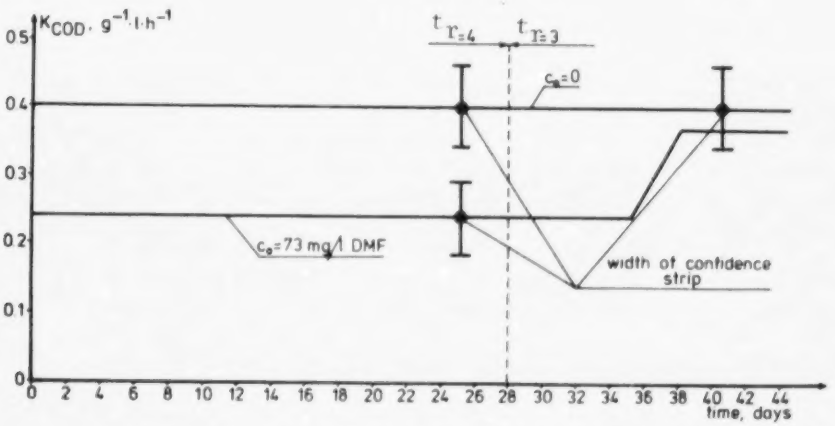


Figure 31. The treatability coefficient K_{COD} of wastewater containing DMF.

TABLE 10. THE CONDITIONS OF RESPIROMETRIC MEASUREMENTS OF PNP BIODEGRADABILITY

No.	Number of samples in the series	Test duration, days	Nutrient medium	Seed	Range of concentration, mg/l
I	5	10	glucose	wastewater	0 - 100
II	5	15	"	"	0 - 100
III	5	5	"	"	0 - 80
IV	6	5	"	"	30
V	6	4	"	"	30
VI	3	3.5	yeast extr.	not adapted wastewater	70
VII	3	3.5	"	adapted wastewater	70

It is visible that 35 days was necessary to acclimatize the activated sludge to DMF. After this period DMF was removed almost completely from the wastewater.

During the acclimatization period, the overall treatability coefficient K_{COD} was much lower than the value corresponding with the control unit, but after this period the K_{COD} value increased and any influence of DMF on the wastewater treatment was not observable.

PARA-NITROPHENOL, PNP

Respirometric Measurements

Seven series of tests were carried out. The conditions of the tests are given in Table 10.

The exemplary curves of BOD and the decrease of the PNP content are given in Fig. 32. The same data, after linearizing transformation are plotted in Fig. 33.

The unit oxygen demand L_u can be read out from Fig. 34 in which the data coming from the second series are plotted.

The mean value, obtained on the basis of all data was equal to: L_u = 1.04 ±0.12 mg O_2/mg PNP.

The amount of developed mineral nitrogen (mainly as NO_3) was equal to ca. 50 percent of the nitrogen bound in PNP.

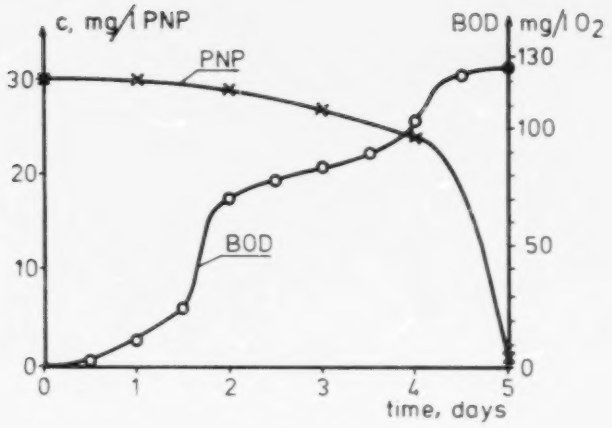


Figure 32. BOD curve and PNP content during the respirometric test.

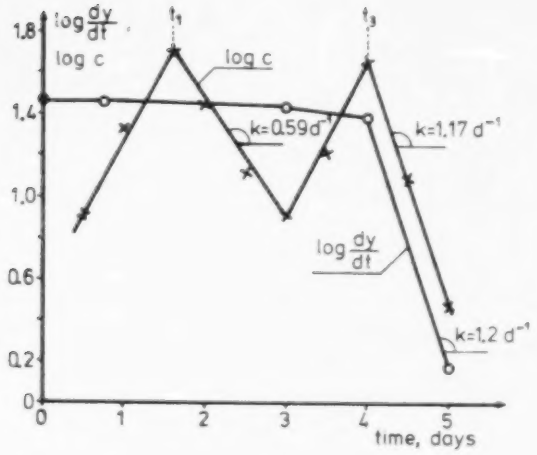


Figure 33. The curves from Figure 32 after linearizing transformation.

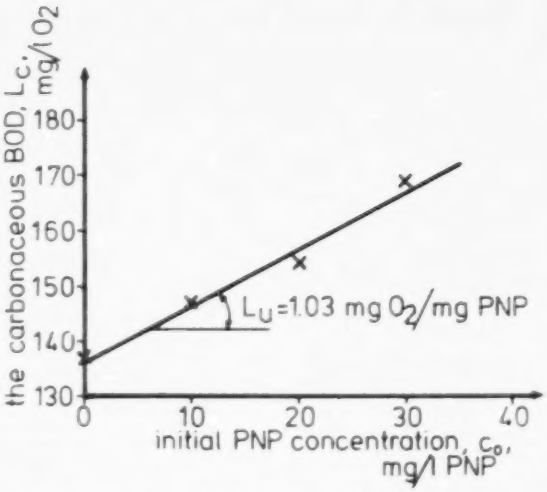


Figure 34. The final carbonaceous BOD L_c as a function of initial PNP content c_o.

The influence of the seed acclimatization on PNP biodegradation is seen in Fig. 35 in which two curves are drawn, corresponding to the samples inoculated with and without acclimatized seed.

The mean values of the inhibition time of the nutrient medium decomposition in the presence of PNP Δt_1 are graphically presented in Fig. 36 as a function of initial PNP content c_o. On the same graph, the mean values of the inhibition time of PNP decomposition Δt_2 are plotted also.

The mean values of the ratio r of the coefficient k_s to the coefficient k_n (corresponding with PNP and nutrient medium biodegradation) are plotted versus the initial PNP content c_o (Fig. 37).

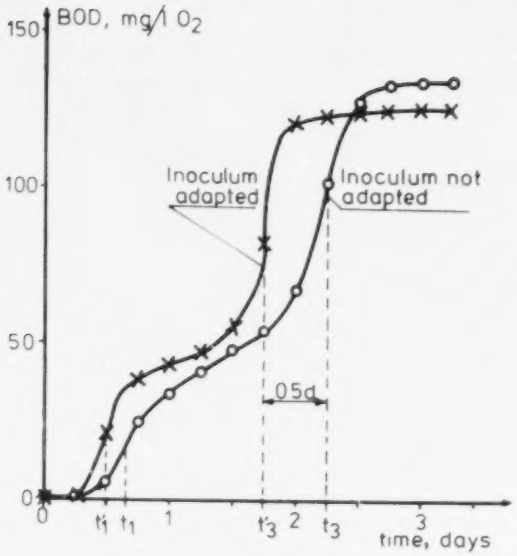


Figure 35. The influence of the seed acclimatization on the biodegradation process.

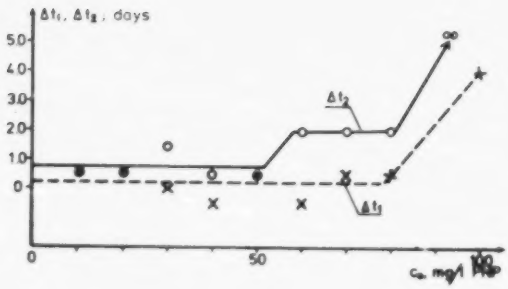


Figure 36. The inhibition time of glucose decomposition Δt_1 and of PNP decomposition Δt_2 as a function of the PNP initial content c_o.

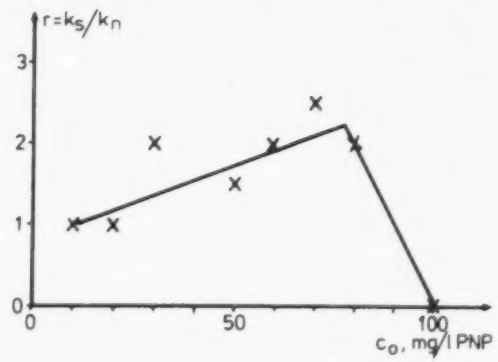


Figure 37. The dependence of the ratio r on the initial PNP concentration c_o.

Discussion

It was found from the experiments that around one half of the nitrogen bound in PNP is converted to nitrates. However, the calculations of unit oxygen demand were based not on the total BOD but on the final carbonaceous BOD which is a more reproducible parameter.

Therefore, the biodegradation of PNP could be expressed by the equation:

$$HO.C_6H_4.NO_2 + 5\ O_2 \longrightarrow 6\ CO_2 + H_2O + NH_3$$

According to this equation, the stoichiometric value of the final carbonaceous BOD equals 1.15 mg O_2/mg PNP. The experimentally determined value (corrected by substracting nitrogen oxygen uptake) was equal to 1.04 ±0.12 mg O_2/mg PNP, i.e. nearly 90 percent of the stoichiometric value. It should be noted that the total BOD of PNP is somewhat higher. If one half of the nitrogen bound in PNP is converted to NO_3 and the rest is assimilated, the experimental unit oxygen demand would be ca. 1.3 mg O_2/mg PNP.

The determination of kinetic parameters allows one to conclude that PNP concentrations up to 80 mg/l inhibits glucose biodegradation just a little. However, at the initial PNP amount c_o = 100 mg/l, the inhibition of glucose biodegradation was distinctly marked (4 days of inhibition period). This proves that at this concentration PNP is toxic for the microorganisms.

At a low PNP content (up to 50 mg/l), the PNP biodegradation proceeds almost simultaneously with the nutrient medium decomposition. At PNP content within the range from 50 to 80 mg/l, the PNP biodegradation was inhibited during 2 days, and at 100 mg/l PNP was almost resistant.

Once the microorganisms are acclimatized to the PNP presence the PNP decomposition proceeds fast. After the inhibition period, which is necessary to microorganisms acclimatization, the ratio r of kinetic coefficients corresponding with PNP and glucose decomposition ranged from 1.0 to 2.5, i.e. PNP was decomposed faster than glucose was.

The previous adaptation of the seed shortens the inhibition time about 0.5 day.

Biodegradation in the river water

Four series of tests were made, each with use of three installations, operating in parallel, everyone with another PNP addition. In every series, PNP was repeatedly added at the moments in which the former PNP dose had been completely decomposed (acclimatization testing).

Figure 38 presents exemplary results of PNP biodegradation in the river water (third series).

On the basis of the data coming from all four series, the mean values of inhibition time t_o and the decomposition velocity v were calculated and are shown in Table 11.

The data in the table show that PNP is easily biodegraded in the river water, especially after the acclimatization of river water biocenosis to the PNP. It was observed that at low concentrations, e.g., 5 mg/l, the PNP biodegradation proceeds more slowly than at the higher concentrations.

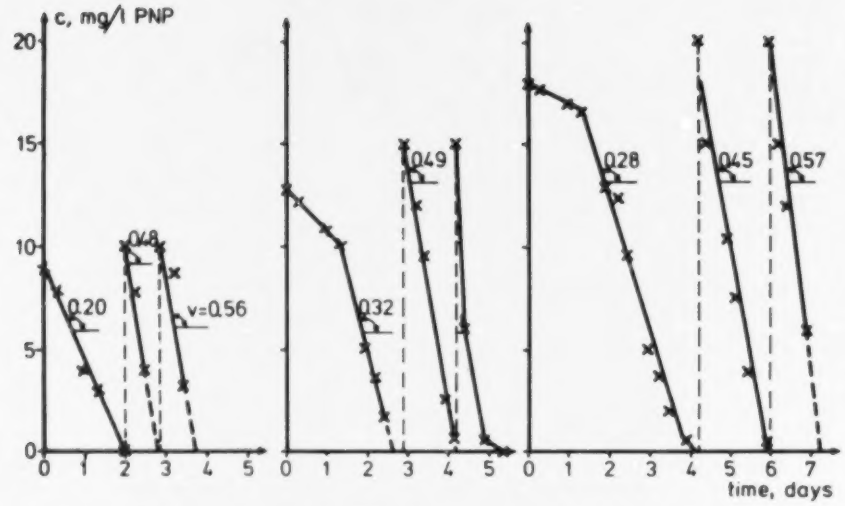


Figure 38. The decomposition of PNP in river water.

TABLE 11. KINETIC PARAMETERS OF PNP BIODEGRADATION IN RIVER WATER

Initial PNP content c_o,	After PNP addition to the fresh river water		After repeated PNP addition		After subsequent PNP addition	
mg/l PNP	v mg/l·h	t days	v mg/l·h	t days	v mg/l·h	t days
5	0.1	0	0.16	0	0.28	0
10	0.18	0.6	0.43	0	0.53	0
15	0.35	1.3	0.48	0	0.50	0
20	0.35	1.4	0.45	0	0.48	0

Treatability Tests

The tests were carried out with use of two activated sludge units operated in parallel. The results of the determinations of PNP removal are presented in Fig. 39. On this graph c_o denotes PNP content in the inflow and c_e denotes PNP content in the effluent.

The curves in the graph show that non-adapted activated sludge removes PNP with low efficiency. After ca. 2 weeks of acclimatization, the PNP removal efficiency becomes high and the effluent contains just traces of PNP.

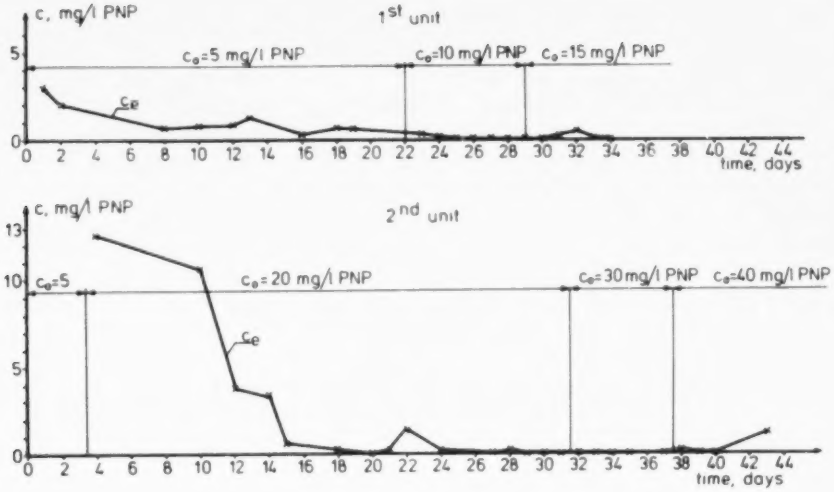


Figure 39. PNP removal by the activated sludge.

The overall treatability coefficient K_{COD} did not depend significantly on the PNP presence in the wastewater. K_{COD} value in the tests units was almost the same as in the control unit. Additional tests were made in which the activated sludge, acclimatized during the former tests, was fed with wastewater containing 200 mg/l PNP. The results showed that after additional three days of acclimatization to such PNP conent, PNP was removed at the efficiency of 90 percent, K_S = 0.7 $g^{-1}l \cdot h^{-1}$.

ORTHO-CHLOROPHENOL, OCP

Respirometric Measurements

Four series of tests were carried out. The first two series consisted in the BOD measurements in the samples containing nutrient medium with various OCP additions ranging 0 - 200 mg/l in the first series and 0 - 1200 mg/l in the second. After these tests were completed, the residual OCP content was determined and was found in all samples as equal to ca. 50 percent of the initial value. It could be caused by biodegradation or by the evaporation of OCP from the solution and by the absorption of OCP vapors by the soda-lime absorbers with which the flasks of Sapromat are equipped. The aim of the third series was to determine this process of evaporation. The samples with the initial OCP content ranging 125 - 1240 mg/l were incubated in Sapromat at the same conditions as formerly, but every day the flasks in Sapromat were open and small samples withdrawn for analyses. The soda-lime mixture was analysed also, but the opening of flasks made the BOD record not feasible. The results of this series showed that all the OCP lost by the samples was found in the soda-lime absorbers, and therefore, no biodegradation of OCP occurred.

The fourth series aimed to determine whether OCP at small concentrations of 5 and 10 mg/l is biodegraded. The method was similar to the procedure

used in the third series: the flasks were open every day and OCP content in the samples and in the soda-lime was determined.

The conditions of the tests performed are described in Table 12. The results of the first and second series enable one to calculate the final carbonaceous BOD L_c of the samples with various OCP content. Exemplary BOD curves are given in Fig. 40 (data coming from the second series). The dependence of L_c on the initial OCP content c_o resulting from these BOD curves is presented in Fig. 41. On the last graph, the points corresponding to c_o = 600 and c_o = 1200 mg/l represent 11 days BOD because it was not possible to determine the final BOD in these cases.

TABLE 12. THE CONDITIONS OF THE TESTS MADE BY MEANS OF SAPROMAT

No.	Number of samples in the series	Test duration, days	Nutrient medium	Seed	Range of concentration, mg/l
I	6	14	yeast extr.	wastewater	0 - 200
II	6	11	" "	"	0 - 1200
III	5	19	" "	"	125 - 1240
IV	11	11	" "	"	0 - 10

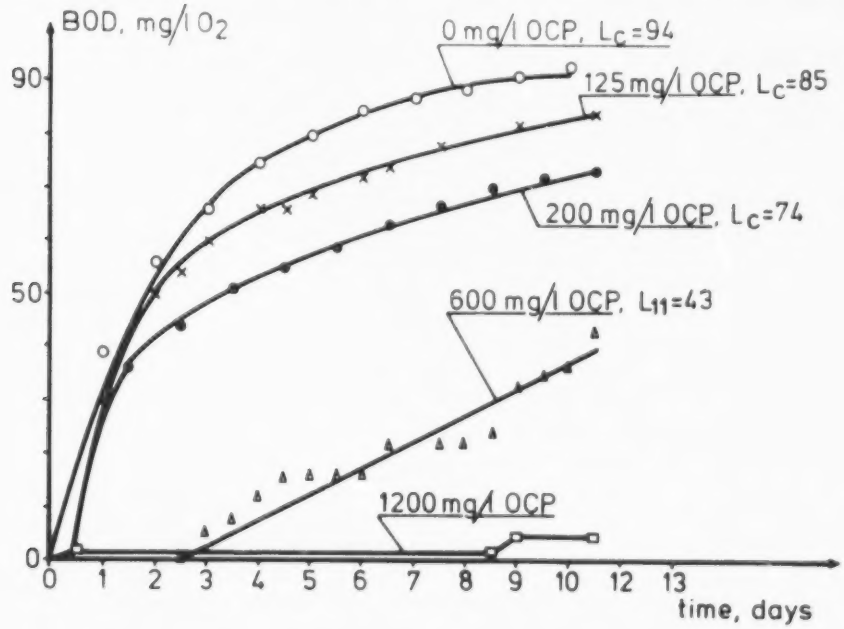


Figure 40. BOD curves of nutrient medium with various concentrations of OCP.

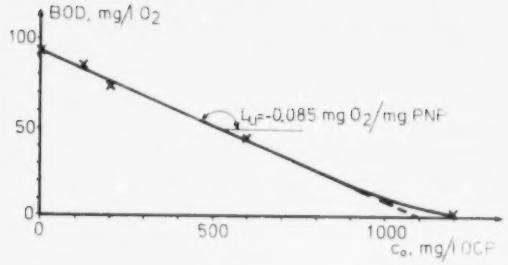


Figure 41. The final carbonaceous BOD L_c as a function of initial OCP content c_o.

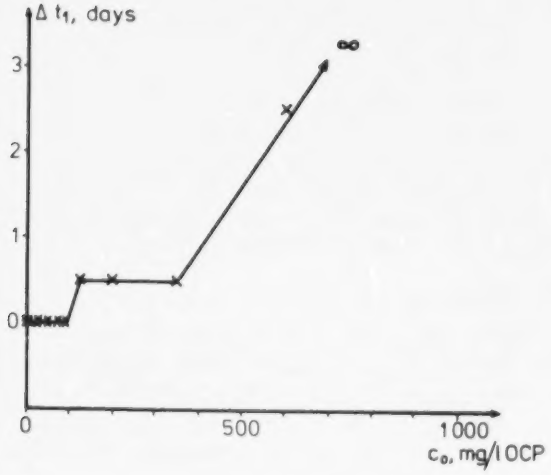


Figure 42. The inhibition time of yeast extract decomposition Δt_1 as a function of the initial OCP content c_o.

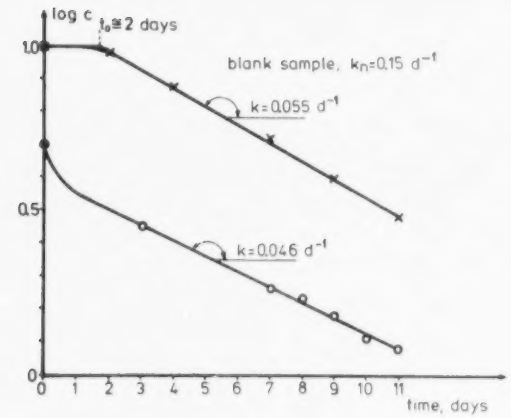


Figure 43. The OCP content decrease during the respirometric test.

The value of the unit oxygen demand L_u read out from Fig. 41 amounts to -0.085 mg O_2/mg OCP. The mean L_u value, calculated on the basis of the first and the second series, amounted to -0.08 mg O_2/mg OCP which means that OCP does not cause any oxygen demand, but it inhibits the process of oxygen uptake for nutrient medium decomposition. Such inhibiting effect is indicated not only by the decrease of final BOD of nutrient medium by the presence of OCP, but also by the delay of nutrient medium biodegradation. The values of this lag duration versus initial OCP content are plotted in Fig. 42.

The tests of OCP biodegradation at low concentrations (5 and 10 mg/1) gave the results presented in Fig. 43. As the BOD was not recorded (except

of blank sample BOD), the curves of OCP content decrease are the only results presented. The OCP concentration found in the soda-lime at the end of the tests are measurable, but it was so small that it could be neglected. The slope of the straight lines on Fig. 43 indicates the value of kinetic coefficient k_s to equal ~0.05d^{-1} which can be compared with the value k_n determined for BOD process in the blank sample, equal to 0.15 d^{-1}. Therefore the ratio $r = k_s:k_n$ is equal to ca. 0.3 in both cases (c_o = 5 and c_o = 10 mg/l). The graph corresponding to 10 mg/l (Fig. 43) shows also a lag phase, lasting ca. 2 days which indicates that the OCP biodegradation needs a certain period for acclimatization.

Discussion

The obtained results show that OCP is only slightly biodegraded in respirometric tests. At its low concentrations, up to 10 mg/l OCP, the decomposition of this substance was observed but the decomposition rate was low. At an initial OCP content of 10 mg/l, two days for microorganisms acclimatization was needed. At higher initial concentrations, OCP is not biodegraded.

However, even at concentrations up to 350 mg/l, OCP just slightly affects the microbiological activity which is expressed by the small decrease of final BOD of nutrient medium and a certain delay of the process beginning. At an initial OCP content of 600 mg/l, the inhibition of the BOD process was distinctly marked, and at 1200 mg/l the BOD process was completely suspended.

Biodegradation in river water

The tests were carried out with the use of four parallel operated installations. The initial OCP content in each of the installations was, respectively: 2, 5, 10 and 20 mg/l OCP. When the OCP content in any of the installations had dropped down to zero, the next OCP dose was added to raise the OCP content to the former initial value. The results of OCP content determinations during these tests are given in Fig. 44.

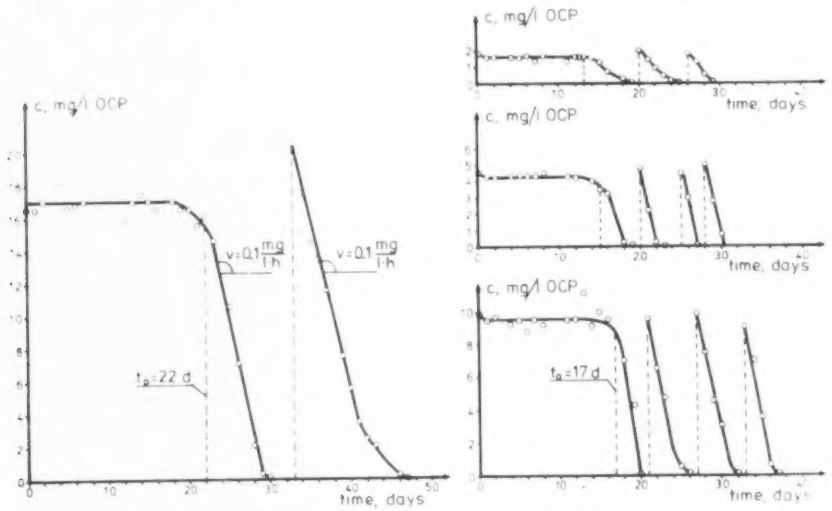


Figure 44. The decomposition of OCP in river water.

TABLE 13. KINETIC PARAMETERS OF OCP BIODEGRADATION IN RIVER WATER

Initial OCP content c_o	After OCP addition to the fresh river water		After repeated OCP addition		After subsequent OCP addition		After subsequent OCP addition	
mg/l OCP	t days	v mg/l·h	t days	v mg/l·h	t days	v mg/l·h	t days	v mg/l·h
2[x]	13	0.02	0	0.02	0	0.03	-	-
5	15	0.07	0	0.10	0.5	0.13	0	0.09
10	17	0.17	0	0.09	0	0.09	0.5	0.13
20	22	0.10	0	0.10	-	-	-	-

[x] At 2 mg/l OCP the decomposition velocity v was not constant. The data in the table are the maximum v values.

The values of parameters read out from Fig. 44 are shown in Table 13. This data led to the conclusion that OCP is biodegraded in the river water but that a long period of acclimatization is needed. This period lasted from 2 to 3 weeks, depending on the initial OCP content. Most probably this microbiological adaptation is a sociological, not physiological one, i.e. that adaptation consists in a change of the species domination in the river water in the presence of OCP. The population of these species which are able to decompose OCP are growing in number during the acclimatization period. Once the biocenosis is adapted to the OCP presence, the decomposition of OCP after the repeated OCP addition begins without any delay. However, even the adapted biocenosis degradates OCP at a low rate, equal approximately to 0.1 mg/l·h. At a low initial content (2 mg/l OCP), the velocity of the decomposition is not constant and it is lower than at the higher concentrations.

Treatability tests

The tests were carried out with use of three parallel operated installations. The results of OCP determinations in the effluent and the determinations of overall treatability coefficient K_{COD} are presented in Fig. 45.

The data given in Fig. 45 show that the activated sludge acclimatization needed 6 to 12 days, depending on the OCP content in the inflow, ranging from 5 to 10 mg/l. In the test in which the non-adapted activated sludge was fed with wastewater containing 20 mg/l OCP, the acclimatization was not reached.

The overall treatability coefficient K_{COD} was the same at 5 mg/l OCP as in the control unit. The adapted activated sludge treated the wastewater

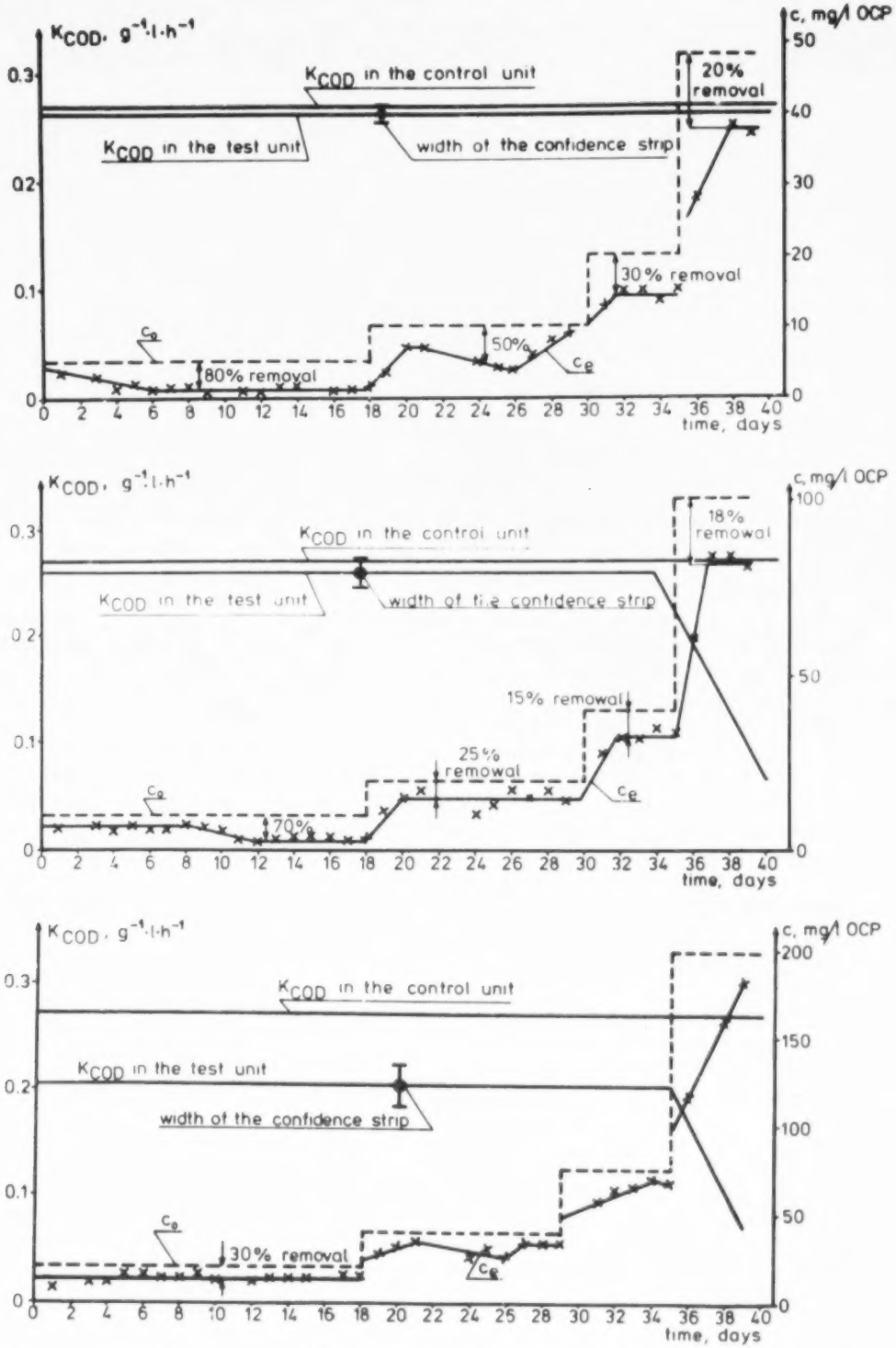


Figure 45. Overall treatability and OCP removal by activated sludge.

with the same value of K_{COD} at OCP concentrations up to 40 mg/l (gradually increased).

Similarly, the feeding of the activated sludge with wastewater with 10 mg/l OCP, gradually increased to 40 mg/l, did not affect the overall treatability. In the test in which the non-adapted activated sludge was fed with wastewater containing 20 mg/l OCP, the value of K_{COD} slightly decreased. However, it remained at the level of ca. 75 percent of the value corresponding with the control unit even when OCP content was increased gradually up to 80 mg/l. OCP at concentrations of 100 mg/l and above poisoned the activated sludge and K_{COD} coefficient decreased down to a very low value.

The OCP removal from wastewater containing up to 10 mg/l OCP proceeds with quite good efficiency $K_S \tilde{=} 0.2\ g^{-1} \cdot l \cdot h^{-1}$. With the increase of OCP content in the inflow, this efficiency decreases and does not depend on the time of the activated sludge acclimatization.

TRICHLOROPHENOL, TCP

Respirometric Measurements

Four series of tests were made. In the first three series, BOD was recorded and the final TCP content was determined. The third series consisted in comparative determinations of the process in the samples seeded with the inoculum alternatively not adapted or previously adapted to the TCP presence. The fourth series consisted in the determination of the TCP content during the test (BOD was not recorded). As TCP is a somewhat volatile substance and because of its acidic properties it can be absorbed by the soda-lime mixture, the determinations of TCP content in the absorbers were also made similarly as in the tests with OCP. The results showed that TCP is much less volatile than OCP. The soda-lime mixture contained measurable quantities of TCP after the tests were completed but these quantities were small and could be neglected.

The conditions of the test performance are given in Table 14.

TABLE 14. CONDITIONS OF RESPIROMETRIC MEASUREMENTS OF TCP BIODEGRADABILITY

No.	Number of samples in the series	Test duration, days	Nutrient medium	Seed	Range of concentration, mg/l
I	8	18	yeast extr.	wastewater	0 - 540
II	6	13	" "	"	0 - 20
IIIa	3	7	" "	not adapted	10
IIIb	3	7	" "	adapted	10
IV	6	7	" "	wastewater	12

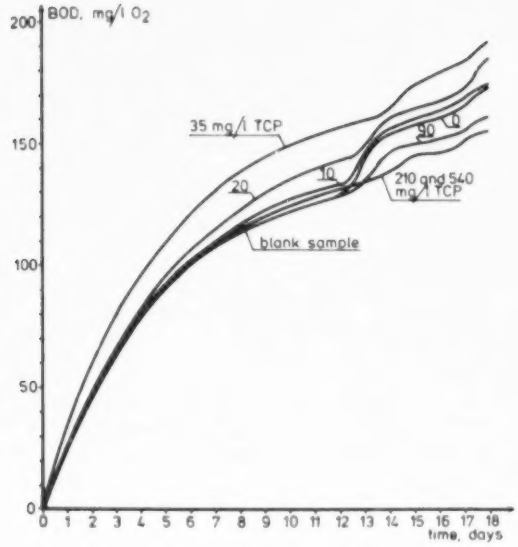


Figure 46. BOD curves of nutrient medium with various concentrations of TCP.

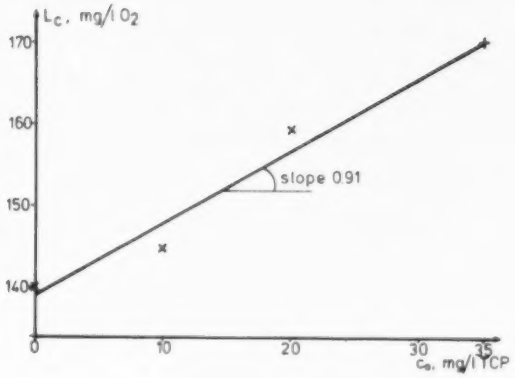


Figure 47. The final carbonaceous BOD L_c as a function of initial TCP content c_o.

TABLE 15. BOD PROCESS IN THE SAMPLE CONTAINING VARIOUS TCP CONCENTRATIONS

Initial TCP content c_o mg/l	Final TCP content mg/l	Final carbonaceous BOD mg/l O_2	NOD mg/l O_2	k d^{-1}
0	0	140	35	0.09
10	0	145	32	0.09
20	0	160	30	0.08
35	0	170	25	0.09
90	80	140	20	0.09
210	190	145	10	0.085
540	500	145	10	0.08

The exemplary BOD curves resulting from the first test are presented in Fig. 46. The logarithmic transformation of those data follows straight lines lying very close to one another. The kinetic parameters read out from the logarithmic curves are presented in more readable tabular form in Table 15.

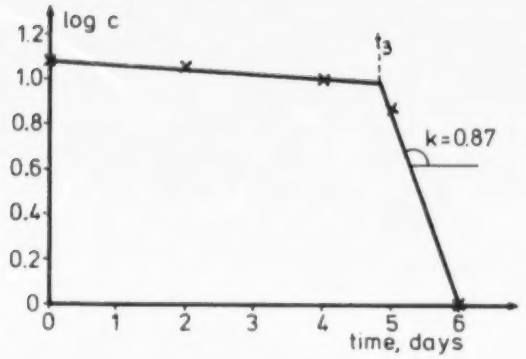


Figure 48. The TCP content decrease at the initial concentration 12 mg/l TCP.

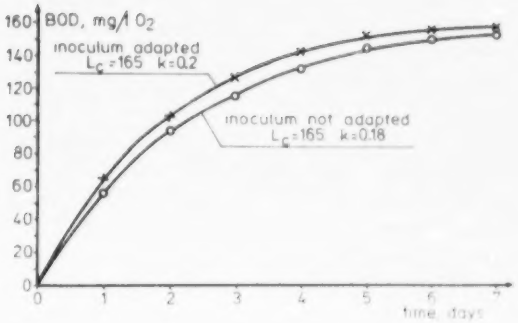


Figure 49. The influence of adaptation on BOD process in the samples containing 10 mg/l TCP.

The dependence of the final carbonaceous BOD of the samples containing nutrient medium and TCP on the initial TCP content c_o is shown in Fig. 47. The points on this graph correspond only with those samples in which TCP was fully decomposed at the end of the tests. This was confirmed by the final TCP content analyses.

The value of unit oxygen demand L_u read out from this graph is equal to L_u = 0.91 mg O_2/mg TCP. The similar determination of L_u in the second series gave the result L_u = 0.85 mg O_2 TCP. These values are not precise because they are based on rather low BOD values of samples with small TCP addition that causes the relative large error.

The test of TCP decomposition in Sapromat apparatus gave the result presented in Fig. 48.

The influence of the seed acclimatization on the BOD process in the presence of 10 mg/l TCP is shown in Fig. 49. The curves are for the seed alternatively adapted and not adapted.

Discussion

If it can be assumed that the TCP biodegradation follows the equation:

$$Cl_3.C_6H_2.OH + 5.5\ O_2 \longrightarrow 6\ CO_2 + 3\ HCl$$

the stoichiometric value of the unit oxygen uptake would amount to 0.89 mg O_2/mg TCP. The mean value resulting from the tests is equal to 0.88 mg O_2/mg TCP which is near to the stoichiometric value. It should be stated that this value is adequate to calculate the final BOD of the samples containing initially 35 mg/l TCP or less. At the higher initial concentration TCP is not biodegraded and does not cause any oxygen demand.

The determination of the kinetic parameters shows that TCP does not inhibit the process of nutrient medium biodegradation. Even at high TCP content of 540 mg/l no delay of nutrient medium decomposition was observed. Similarly the value of k_n coefficient and the final BOD of nutrient medium were not decreased by the presence of TCP.

The TCP decomposition proceeded only at the initial TCP concentration up to 35 mg/l. Any delay of the TCP decomposition could not be read out from the BOD curves. However, the curve of TCP content decrease (Fig. 48) indicates that TCP decomposition at its initial contents 12 mg/l needed ca. 5 days for microorganisms acclimatization. After that the TCP decomposition proceeded at a high rate ($k_s \cong 0.9\ d^{-1}$).

The previous acclimatization of the seed to the TCP presence accelerates the BOD process. This is indicated by some increase of the coefficient k value (Fig. 49).

Biodegradation Tests in River Water

Two series of the tests were made. In the first series two river models were operating in parallel with the initial TCP content 8 and 15 mg/l, respectively. The second series was carried out with use of three river models with initial TCP content 5, 8 and 10 mg/l. In any case, when TCP was fully decomposed, the TCP dose was repeated (the tests of microorganisms acclimatization to the TCP presence).

The results of both series are presented in Fig. 50. These graphs show, that the TCP biodegradation in the river water needs a certain period of biocenosis acclimatization (ca. 6 days) during which TCP is decomposed at a low rate. Once the biocenosis is acclimatized, the next dose of TCP is biodegraded without any delay (or after short delay) and at a rate of about 0.35 mg/l·h. At low initial TCP content (5 mg/l), this rate is somewhat lower.

Treatability Tests

One test lasting 38 days was carried out. The activated sludge was fed with wastewater with gradually raising the TCP content from 5 mg/l at the test beginning to 25 mg/l at the end of the test.

The results of the determinations of the TCP in the effluent c_e and of the overall treatability coefficient K_{COD} are presented graphically in Fig. 51. On the basis of these results, the following conclusions can be made. The activated sludge did not need any acclimatization period for its normal activity until the TCP in the inflow surpassed 18 mg/l. The overall treatability coefficient K_{COD} was stable during the tests and equal the value of K_{COD} corresponding to the control unit. However, when the TCP content in the inflow equaled 25 mg/l it poisoned the activated sludge, and K_{COD} dropped to zero.

The TCP removal from wastewater needs some period of adaptation t_1 depending on the TCP content in the inflow: at 5 mg/l TCP $t_1 \cong 0$; at

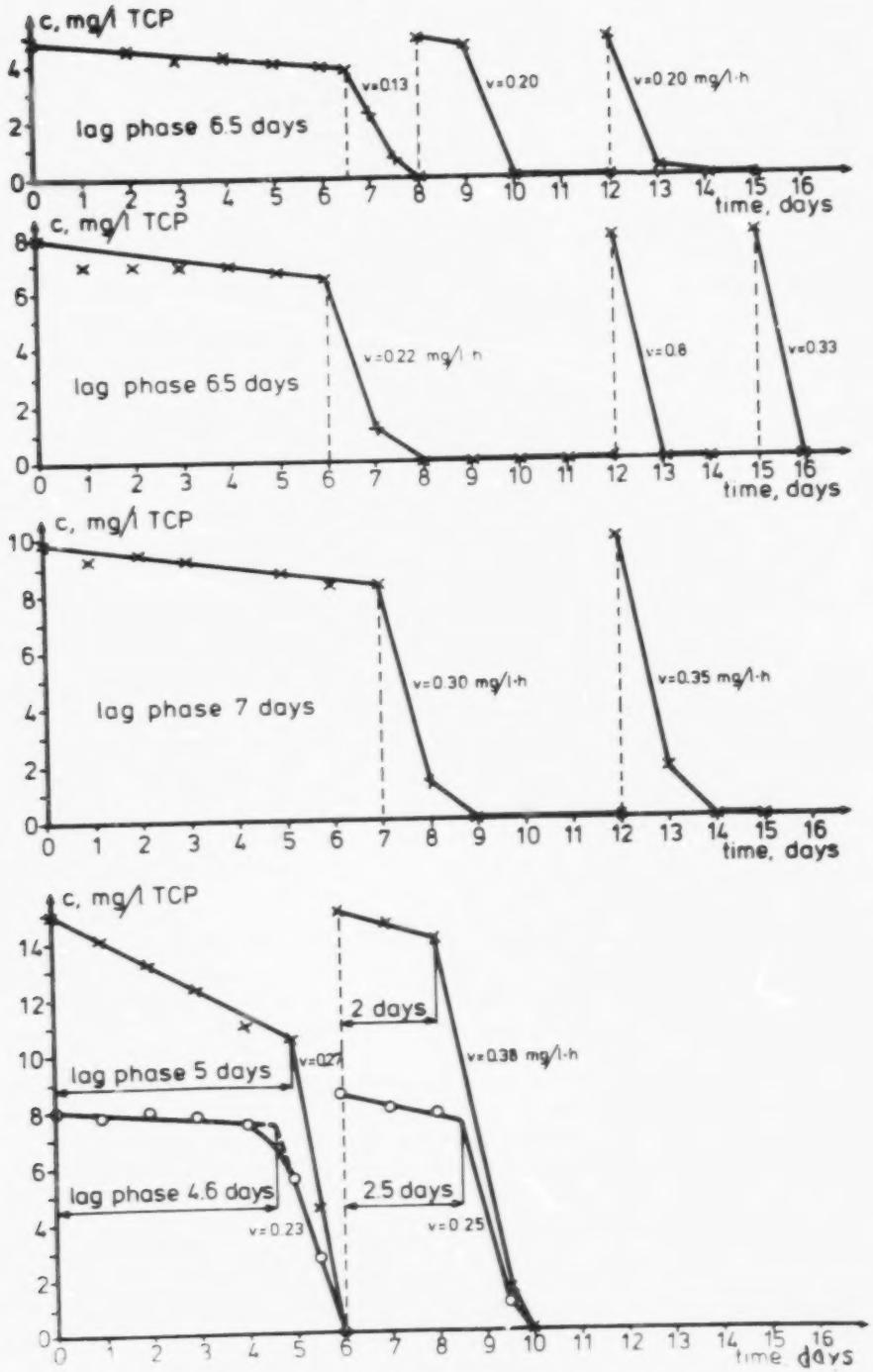


Figure 50. The decomposition of TCP in river water.

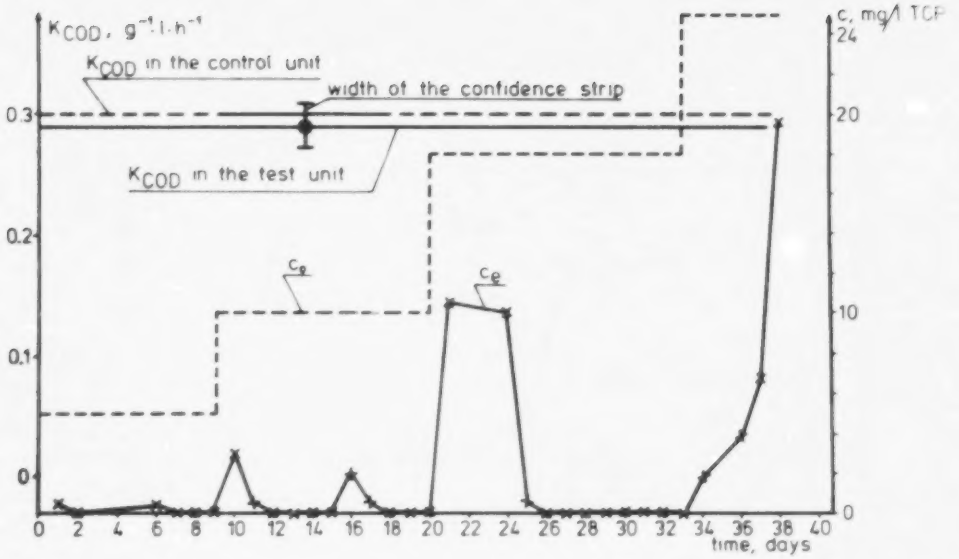


Figure 51. Overall treatability and TCP removal by activated sludge.

10 mg/l TCP $t_1 \cong 2$; at 18 mg/l TCP $t_1 \cong 5$ days. The adapted activated sludge removed TCP from wastewater with high efficiency; the effluent contained just traces of TCP. An increase of TCP contents in the inflow up to 35 mg/l caused a decrease of TCP removal efficiency down to near zero.

DICHLORODIETHYL ETHER, DCDEE

Respirometric Measurements

Two series of the tests were carried out. Yeast extract (200 mg/l) was used as nutrient medium and the samples were seeded with the wastewater previously aerated. The DCDEE contents ranged from 0 to 500 mg/l in the first series and from 0 to 15 mg/l in the second one.

Exemplary BOD curve from the first series is presented in the Fig. 52. All BOD curves obtained in this series were posed very close one to another. Therefore, to make the graph more readable Fig. 52 shows only the BOD curve of the sample containing 100 mg/l DCDEE, compared with the BOD curve of blank sample and the carbonaceous BOD of the blank sample, corrected on the basis of NO_2 and NO_3 content determinations.

The values of final carbonaceous BOD were determined for every BOD curve and they are plotted versus the initial DCDEE content (Fig. 53). The slope of the straight line in Fig. 53 does not differ significantly from zero. It means that the unit oxygen uptake caused by DCDEE is equal to zero or too small to be measurable.

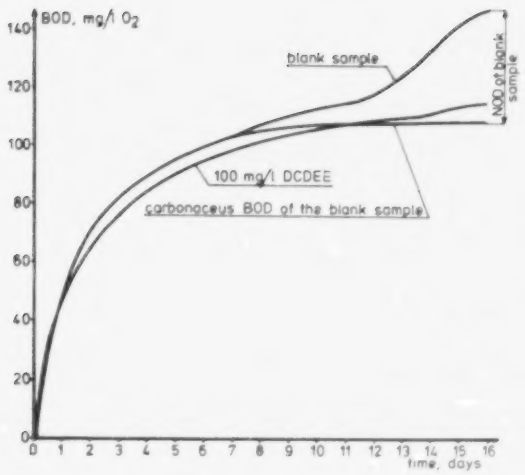


Figure 52. BOD curve of the sample containing DCDEE.

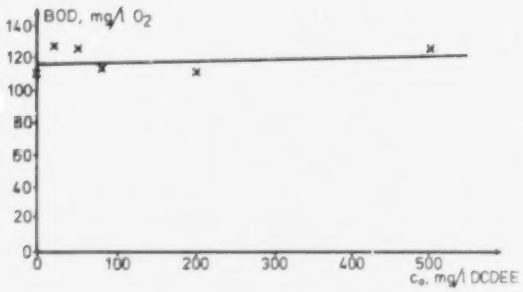


Figure 53. Final carbonaceous BOD L_c as a function of initial DCDEE content c_o.

The values of k_n coefficient of nutrient medium biodegradation ranged irregularly within the narrow limits 0.15 - 0.17 d^{-1} in the first series and 0.2 to 0.25 d^{-1} in the second series. The lag phase of nutrient medium biodegradation was not observed in any test.

The final DCDEE content and the NO_2 and NO_3 increase during the tests are given in Table 16. In the second series no change of DCDEE content during the test was observed either.

TABLE 16. THE INITIAL AND FINAL DCDEE CONTENT AND NO_2 AND NO_3 INCREASE DURING THE TESTS IN SAPROMAT (THE FIRST SERIES)

DCDEE		$NO_2 + NO_3$
Initial c_o, mg/l	Final c_f, mg/l	mg/l N
0	0	9.5
14	15	1.2
41	40	0.5
107	109	0.3
205	203	0.2
512	501	0.6

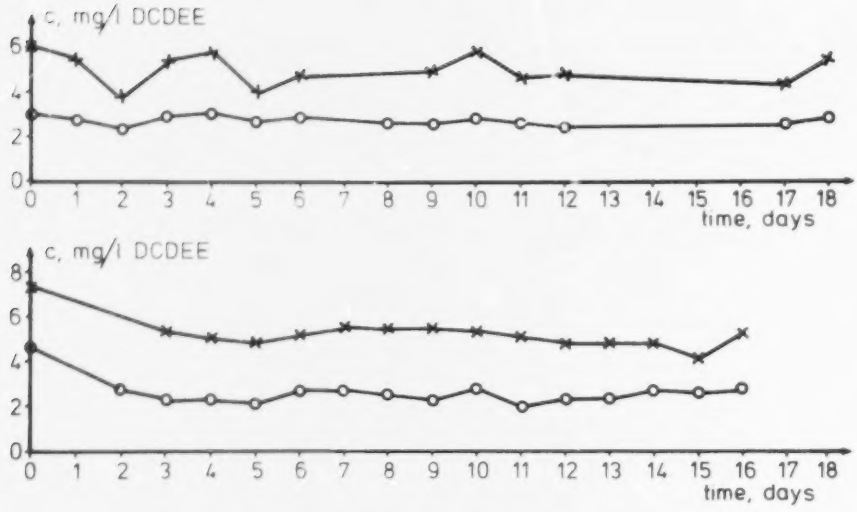


Figure 54. DCDEE changes in river water.

Discussion

The unit oxygen uptake caused by DCDEE was measured as equal to zero ($L_u = 0$). No lag phase of nutrient - medium decomposition was observed. The coefficient k_n of nutrient medium decomposition was also not affected by the DCDEE. The determination of the final DCDEE content showed that DCDEE is not degraded during the tests. Summarizing, it can be concluded on the basis of these results that DCDEE is an inert substance which is not biodegraded but does not affect in anyway the biodegradation of the other organics (yeast extract components).

The only effect of DCDEE on the biochemical processes was the inhibition of nitrification. At a DCDEE concentration of 14 mg/l and higher, the nitrification was almost completely inhibited; whereas, in the blank sample almost all the ammonia was converted to NO_2 and NO_3.

Biodegradation in River Water

Two series of tests were made using two units operating in parallel. As DCDEE is a volatile substance, the river models were sealed to avoid any DCDEE loses due to evaporation. In both series the DCDEE content was analyzed during the tests. The results are presented in Fig. 54.

In the second series the NH_3 and NO_2 changes were also observed during the test. The aim was to determine the influence of DCDEE on the nitrification of ammonia in river water. The results are presented in Fig. 55.

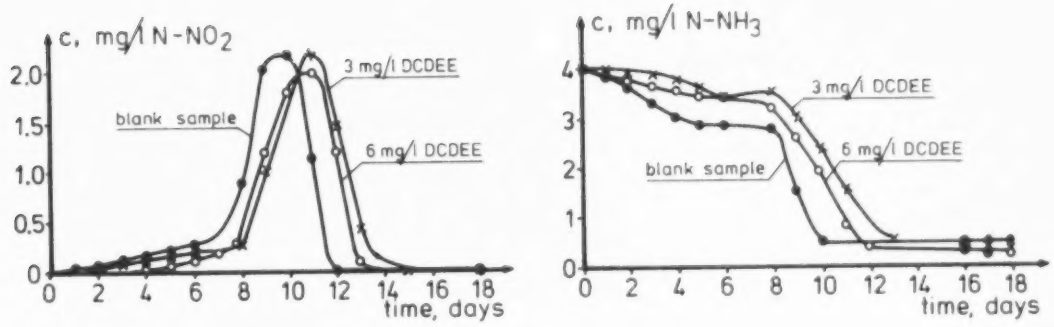


Figure 55. Nitrites and ammonia changes in river water.

Discussion

The DCDEE content curves show that this substance is resistant in the river water. A small decrease of DCDEE was observed at the beginning of tests. Beyond this, no further DCDEE decrease was observed.

The observation of the nitrification process leads to the conclusion that DCDEE at concentrations up to 6 mg/l does not inhibit the nitrification but causes a delay of this process (lasting 1.5 to 2 days).

Treatability Tests

One series of test was carried out by use of two parallel operating activated sludge units fed with the wastewater containing 5 and 10 mg/l DCDEE, respectively. As DCDEE is a volatile substance, the supplementary determination of its Henry coefficient $\underline{a}$ was made. The air was bubbled through the DCDEE solution in distilled water. DCDEE content and the weight of the solution were measured during the test. The results were plotted in the logarithmic coordinates system (Fig. 56). The slope of the straight line was read out, and according to the equation given in Section 6, the value of coefficient $\underline{a}$ was calculated equal to 0.37×10^{-3}. Assuming that the evaporation of DCDEE is the only cause of its content decrease in the activated sludge chamber, the DCDEE content in the effluent would be equal to:

$$c_e = \frac{c_o}{1+a \cdot \frac{V_a}{V_w}}$$

where V_a is the rate of air blowing, equal to 300 l/h and V_w is the rate of wastewater inflow, equal to 0.17 l/h. As the value of $\underline{a}$ amounts to 0.37×10^{-3}, c_e should be equal to $0.6 \cdot c_o$.

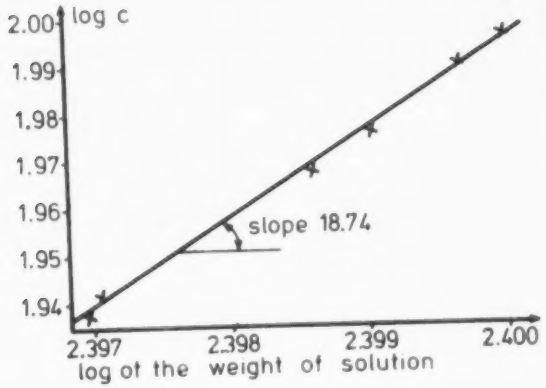


Figure 56. The volatility of DCDEE from aqueous solution.

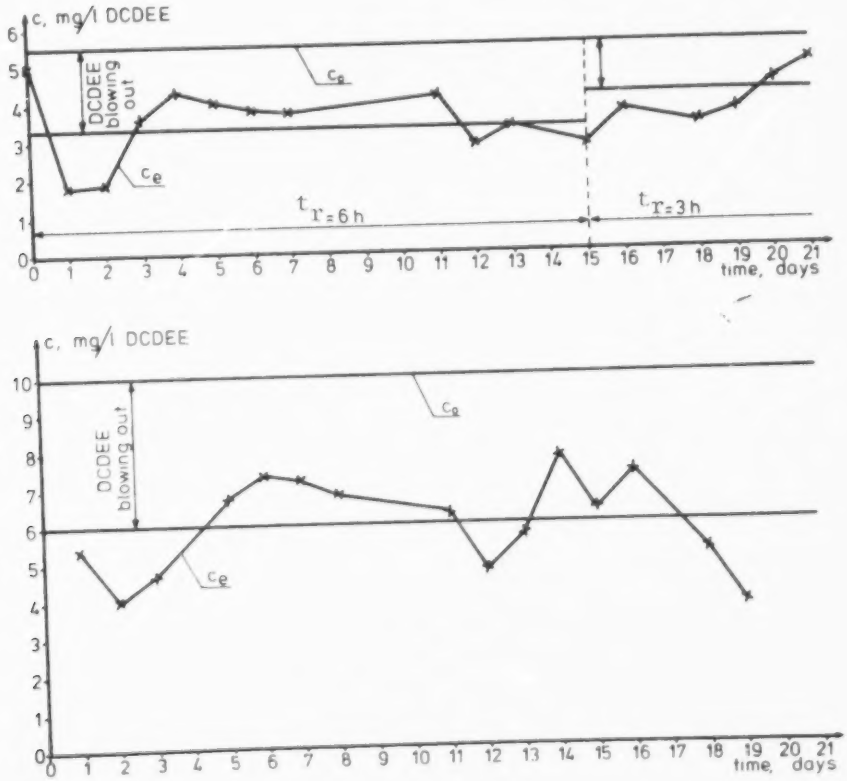


Figure 57. DCDEE concentration in the effluent from activated sludge unit.

The results of the determinations of the DCDEE content in the effluent c_e are presented in Fig. 57 on which the theoretical DCDEE content decrease by the blowing out is also marked.

The overall treatability coefficients K_{COD} were calculated on the basis of COD determinations. The obtained values show just irregular scattering. The mean values and their confidence limits are as below:

control unit $K_{COD} = 0.20 \pm 0.06$

at DCDEE content in the inflow of 5 mg/l, $K_{COD} = 0.20 \pm 0.07$

at DCDEE content in the inflow of 10 mg/l, $K_{COD} = 0.16 \pm 0.09$

The scattering of the results causes rather wide confidence limits. Because of it, any decrease of K_{COD} value in the presence of DCDEE cannot be stated.

It can be concluded that DCDEE content in wastewater does not affect significantly the overall treatability of wastewater (i.e. the COD decrease). Thus, DCDEE is an inert substance in the treatment process. Its removal is caused just by the blowing off with the air in the aeration chamber.

FLUORESCENT WHITENING AGENTS, FWAs

Respirometric Measurements

Two kinds of the tests were carried out. The tests of the first kind (BOD tests) consisted in the recording of the BOD of the samples, each containing nutrient medium and one of the five various FWAs (100 mg/l). For the inoculum, the activated sludge supernatant was used. After completing the tests, lasting 12 days, the final FWAs content was determined. Next the tests were repeated, similarly as before, except that the solutions remaining from the previous tests were used as inoculum (acclimatization tests).

The tests of the second kind consisted in the measurement of the activated sludge endogenous respiration in the presence of various FWAs. The procedure was as follows: the settled activated sludge was put into the flasks of Sapromat and the FWAs solutions in the dilution water were added. The activated sludge respiration was recorded during 10 days and compared with the respiration of the blank sample (the same activated sludge without any FWA addition). Every FWA was tested in three identical samples to reach the higher reliability of the results. After the tests were finished, the FWAs content were determined.

The results of the BOD test for FWA-1 is presented in Fig. 58. The BOD curves for the other FWAs are very similar to that shown in Fig. 58, e.g., the blank sample and the sample with 100 mg/l FWA have identical oxygen uptake. Figs. 59 and 60 present exemplary curves of the activated sludge endogenous respiration in the presence of FWA-1 and FWA-3.

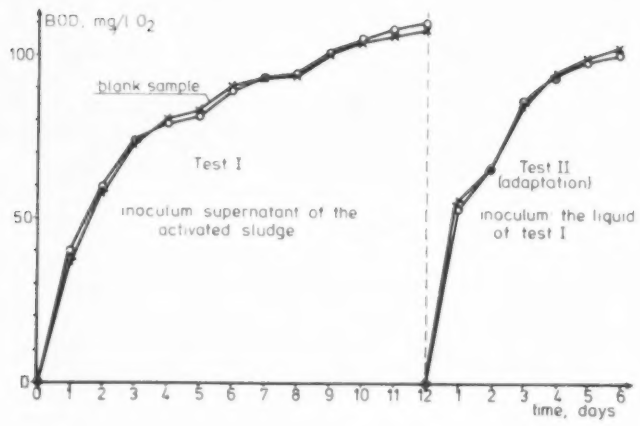

BOD, mg/l O2
100
50
0
blank sample
Test I
inoculum: supernatant of the activated sludge
Test II (adaptation)
inoculum: the liquid of test I
0 1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6
time, days

Figure 58. BOD curves of the samples containing nutrient medium and 100 mg/l of FWA-1.

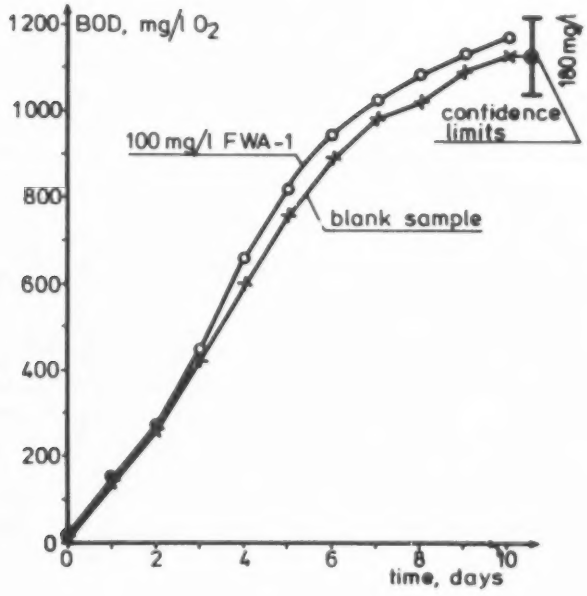


Figure 59. The curve of activated sludge endogenous respiration in the presence of FWA-1.

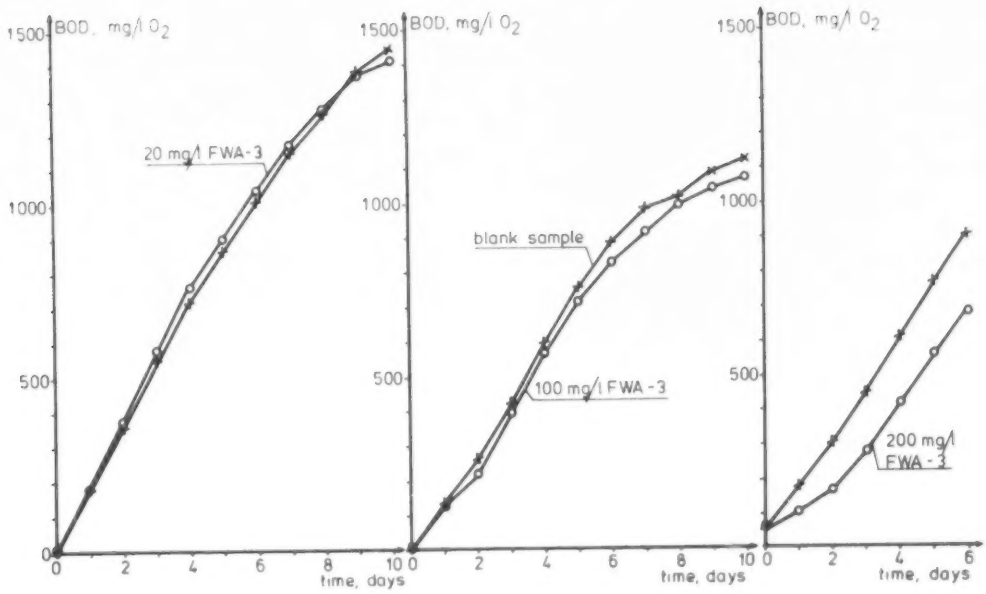


Figure 60. The curves of activated sludge endogenous respiration in the presence of FWA-3.

TABLE 17. THE FINAL FWAs CONCENTRATIONS IN THE SAMPLES CONTAINING INITIALLY ONE OF THE FIVE FWAs (100 mg/l). (THE TESTS LASTED 10 DAYS.)

Final FWA concentration mg/l	FWA-1	FWA-2	FWA-3	FWA-4	FWA-5
	5	21	91	69	87

The results of final FWAs content determination are summarized in Table 17 (BOD tests) and in Table 18 (activated sludge respiration tests). The last table contains also the data of final BOD (mean values and the limits at 90 percent confidence).

TABLE 18. BOD AND FINAL FWA CONTENT IN THE SAMPLES CONTAINING CA. 2 g/1 OF ACTIVATED SLUDGE AND ONE OF THE FWAs.

FWA	Initial concentration mg/1	Final concentration mg/1	BOD_{10} mg/1 O_2
FWA-1	0	0	1125 ± 130
	100	5	1170 ± 90
	0	0	1500 ± 140
	100	10	1520 ± 110
FWA-2	180	15	1610 ± 100
	300	30	1780 ± 180
	0	0	1450
	20	4	1400
FWA-3	0	0	1130 ± 120
	100	70	1080 ± 100
	0	0	900 ± 80
	200	180	680 ± 30
	0	0	920 ± 150
FWA-4	20	15	925 ± 110
	100	70	1060 ± 190
	0	0	1530 ± 180
FWA-5	20	10	1550 ± 120
	100	100	1440 ± 80

Discussion

The BOD curves of the samples containing nutrient medium and FWAs additions show that the FWAs do not affect the BOD process. It means that the FWAs are not subject to very much decomposition, which would cause the oxygen uptake, and that they do not inhibit the process of nutrient medium biodegradation. Acclimatization of microorganisms to the FWAs presence does not change the BOD processes.

The similar conclusion can be made on the basis of the activated sludge respiration data. The oxygen uptake by activated sludge endogenous respiration in the FWAs presence was almost the same as in the blank sample

(the differences were statistically not significant). One exception was the FWA-3 at 200 mg/l which inhibited the respiration of activated sludge.

The data of final FWAs contents show that FWA-1 and FWA-2 are not resistant at the conditions of the tests (i.e. in the samples aerated and kept in darkness). The final FWA-4 content was not much lower than its initial content. The FWA-3 and FWA-5 were almost resistant in the solutions with their high initial contents. But in the case of FWA-3 at the low initial content equal to 20 mg/l, it decreased to a low value. It may be caused by the FWA-3 adsorption on the activated sludge flocs because this FWA is easily adsorbed as it was confirmed in the experiments described further.

It should be underlined that the above described partial FWAs decomposition was based on fluorimetric analyses. The FWAs concentrations were determined based on the fluorescent properties of FWAs molecules. Such results do not mean that the FWAs are really degraded. The FWAs molecules could lose their fluorescent properties as a result of a simple transfiguration (e.g., cis-trans) or as a result of their breaking into large fragments (e.g., by the oxidation and breaking of the double bond of styrene structure). As no measurable oxygen uptake was observed in the samples in which FWAs content decreased, it can be concluded that no large amount of degradation occurred.

The Decomposition in River Water

Two kinds of tests were made. The tests of the first kind consisted in the long term observation of the FWAs content changes in river water kept in darkness. In parallel, FWAs in distilled water, kept at the same conditions, were also observed.

In the tests of the second kind, the river water with FWAs was exposed to the light of fluorescent tubes at an intensity of 1000 lux. Also in these tests, FWAs solutions in the distilled water, exposed to the light, were used as the blank samples. In the last case, the total organic carbon (TOC) was also measured. Additionally, the river water models and blank samples were kept in the darkness to compare the FWAs decomposition in the presence and in the absence of the light.

As the rate of the FWAs photolytic decomposition depends on pH, phosphate buffer solution was always added to the blank samples (FWAs solutions in the distilled water) to keep pH at the same level as pH of river water used in the tests (8 - 8.5).

The results of 30 days testing of the FWAs fate in the river water in the absence of the light showed that FWAs at these conditions are almost resistant. The FWAs contents decreased just a little, 10 to 20 percent of the initial value (10 mg/l). Even this small decrease probably was not related to the biodegradability because in the blank samples, which were almost microbiologically sterile, some FWAs decrease was observed.

The decomposition of FWAs exposed to light proceeded at a high rate so that FWAs content had to be measured every half hour. In the samples

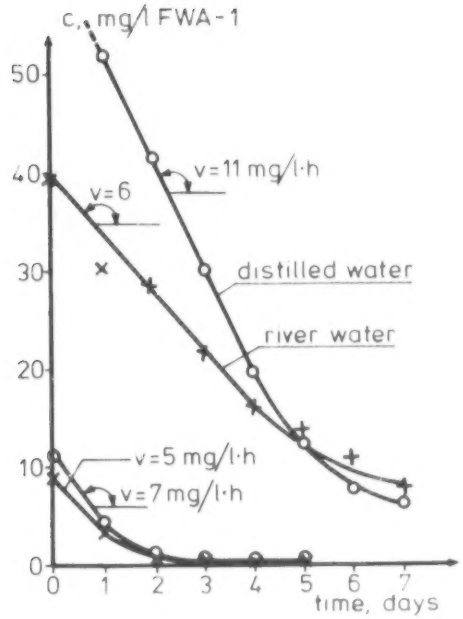


Figure 61. Photolytic FWA-1 decomposition in river and distilled water.

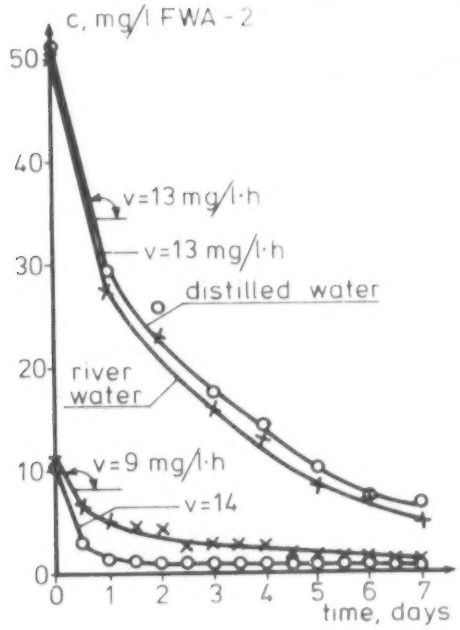


Figure 62. Photolytic FWA-2 decomposition in river and distilled water.

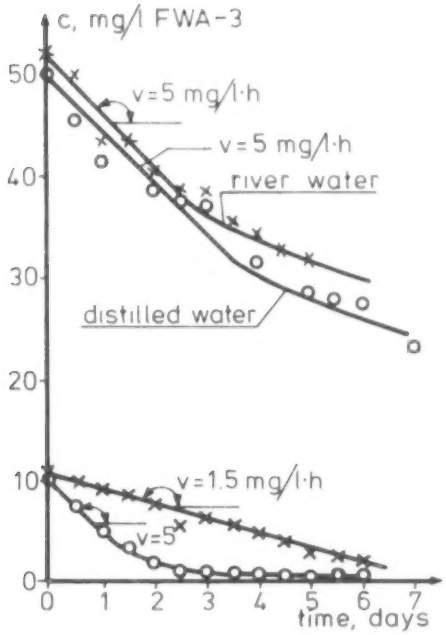


Figure 63. Photolytic FWA-3 decomposition in river and distilled water.

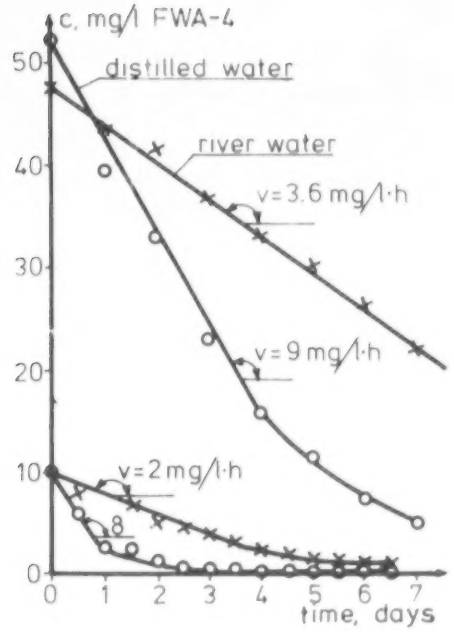


Figure 64. Photolytic FWA-4 decomposition in river and distilled water.

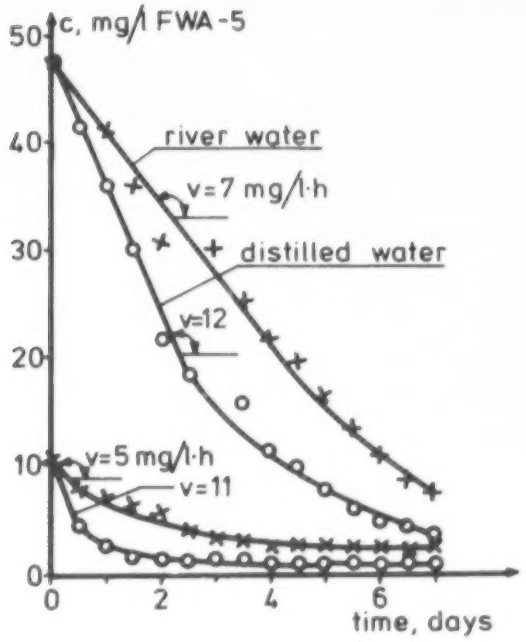


Figure 65. Photolytic FWA-5 decomposition in river and distilled water.

TABLE 19. VELOCITY v OF THE FWAs DECOMPOSITION IN RIVER AND DISTILLED WATER EXPOSED TO LIGHT

FWA	Velocity of FWAs decomposition, mg/l·h			
	In river water at initial FWA content		In distilled water at initial FWA content	
	c_o = 10	c_o = 50	c_o = 10	c_o = 50
FWA-1	5	6	7	11
FWA-2	9	13	14	13
FWA-3	1.5	5	5	5
FWA-4	2	4	8	9
FWA-5	5	7	11	12

simultaneously kept in darkness no FWAs change was observed. The curves of FWAs decomposition are presented in Figs. 61, 62, 63, 64 and 65.

The curves show the decrease of FWA down to the residual value c_R related most probably to the fluorescence of the FWA decomposition product.

During the initial period of FWAs photolysis, the decomposition velocity is usually constant. The values of decomposition velocity v, being equal to the slope of the initial section of lines, are read out from the graphs and are shown in Table 19.

The additional TOC determinations in the samples of FWAs in distilled water show that the FWAs decomposition is not related to any TOC decrease. Final TOC values were almost the same as the initial values, whereas the FWAs content dropped down to the residual values c_R.

Discussion

The long term tests of the FWAs fate in the river water in the absence of light have shown that all FWAs tested are resistant. No FWAs biodegradation was observed during 30 days. The observed small FWAs decrease, equal to 10 to 20 percent of the initial FWAs concentrations, is probably due to physical processes.

The tests of FWAs decomposition in the presence of the light have shown that all FWAs tested are subject to the photolytic decomposition. According to the tests with FWAs solutions in distilled water, the most easily decomposed FWAs are FWA-2 and FWA-5, whereas FWA-3 is the most difficult to decompose with a rate less than one half of the FWA-2 or FWA-5 decomposition rate.

The decomposition rate is initially almost not dependent on the FWAs concentration and next it is decreasing asymptotically to zero. The FWAs solutions after a long time of exposition to the light (1 days or more) retain a residual fluorescence, corresponding with 5 to 10 percent of their initial value.

The decomposition of FWAs in river water proceeded similarly as in the distilled water. At the initial FWAs concentration of 50 mg/l, the decomposition rate in river water was equal to or less than in the distilled water. At the concentration of 10 mg/l, the FWAs were decomposed in river water at significantly lower rates. The fact that the decomposition of FWAs is slower in the river water than in the distilled water can be explained as a result of the light extinction in the river water because of colored impurities. This absorption of the light decreases the amount of light needed for photochemical reaction of FWAs decomposition.

The fact that the solutions keep the residual fluorescence and that the TOC is not changed during the tests, leads to the conclusion that chemical changes of FWAs molecules were very little. It could be a cis-trans transfiguration or division to large fragments.

TABLE 20. THE ADSORPTION OF FWAs ON ACTIVATED SLUDGE

	c_o	c_e	m	a	b
FWA-1	5	1.72	4.8	0.6	1.4
	10	4.86			
FWA-2 test 1	5	3.52	6.9	3.1	5.6
	10	7.96			
test 2	5	3.45	7.5		
	10	8.26			
FWA-3 test 1	5	1.5	6.6	1.0	1.0
	10	5.1			
test 2	5	0.9	7.3		
	10	3.75			
FWA-4	5	3.72	4.8	1.3	9.2
	10	8.03			
FWA-5	5	5	7.3	∞	∞
	10	10			

Treatability Tests

As the adsorption of FWAs on the flocs of activated sludge can interfere with the FWAs biodegradation determinations, supplementary tests of FWAs adsorption were carried out. The procedure was as follows: into the sample of the settled activated sludge of a known density (dry mass) the FWAs stock solution was added and after 1 hour of FWA contact with the activated sludge the FWA content in the supernatant was determined. Next, the coefficients of the Langmuire's isotherm of adsorption were calculated, following the equation:

$$\frac{c_o - c_e}{m} = \frac{c_e}{a \cdot c_e + b}$$, where:

c_o is the initial FWA content in the supernatant, mg/l

c_e is the FWA content in the supernatant at the state of equilibrium with the adsorbed FWA, mg/l

m is the dry mass of the activated sludge, g/l

a and b are Langumire's coefficients.

The results are summarized in Table 20.

The tests of the FWAs treatability were carried out with the use of three parallel operated activated sludge units as follows:

- test unit, fed with wastewater containing the FWA addition
- control unit I, fed with wastewater without any FWA addition
- control unit II, fed with distilled water with FWA addition.

The measured parameters were:

- FWAs content in the inflow c_o and in the effluent c_e
- COD in the inflow and in the effluent
- activated sludge dry mass $\underline{m}$ in the test unit and control unit I.

On the basis of the obtained data, the following treatability coefficients were calculated. The general FWAs removal coefficient K was determined by use of the equation

$$K = \frac{c_o - c_e}{c_e m t_r}$$

where t_r is the retention time. It can be assumed that the general coefficient K is the sum of the coefficient K_b of FWA removal by degradation, the coefficient K_{ph} of FWA removal by photolysis and the coefficient K_{ad} of FWA removal by adsorption on the sludge. The values K_{ph} were determined by use of the equation

$$K_{ph} = \frac{\Delta c_{II}}{c_e} \cdot \frac{1}{m t_r}$$

where Δc_{II} is the FWA decrease in the test unit II; c_e, m and t_r are corresponding with the test unit. The values of K_{ad} were calculated on the basis of formerly determined Langumire's coefficients:

$$K_{ad} = \frac{1}{\Theta} \cdot \frac{1}{a \cdot c_e + b}$$

where Θ is the sludge age equal to 120 hours, a and b are Langumire's coefficients (Table 20), c_e is FWA content in the effluent from the test unit. The values of the coefficient of FWAs removal by the biodegradation are equal to $K_b = K - K_{ph} - K_{ad}$.

The overall treatability coefficient K_{COD} was calculated by use of the equation

$$K_{COD} = \frac{COD_o - COD_e}{COD_e} \cdot \frac{1}{mt_r}$$

In cases when the tests were made at high FWAs concentrations in the inflow, the K_{COD} values were corrected by substracting the value of COD corresponding with FWA content both from the measured COD of the inflow and of the effluent. This corrected value is denoted as K'_{COD}.

The results of subsequent FWAs tests are presented in Tables 21, 22, 23, 24 and 25. In the case of FWA-1, the photolysis significantly decreased FWA content in the effluent; the general coefficient of FWA removal was approximately twice the value of K_b. In the case of FWA-2, the photolysis caused a small decrease of FWA content. Practically, it was the only cause of FWA removal.

TABLE 21. THE TREATABILITY OF FWA-1

Test duration	FWA-1 in the inflow	FWA-1 in the effluent	FWA-1 biodegradation coefficient K_b	Overall treatability coefficients	
				test unit K'_{COD}	control unit K_{COD}
days	c_o mg/l	c_e mg/l	$g^{-1} \cdot l \cdot h^{-1}$	$g^{-1} \cdot l \cdot h^{-1}$	$g^{-1} \cdot l \cdot h^{-1}$
20	9.9	1.6	0.08	0.17	0.17
27	40.0	8.0	0.10	0.17	0.17

TABLE 22. THE TREATABILITY OF FWA-2

Test duration	FWA-2 in the inflow	FWA-2 in the effluent	FWA-2 biodegradation coefficient K_b	Overall treatability coefficients	
				test unit K'_{COD}	control unit K_{COD}
days	c_o mg/l	c_e mg/l	$g^{-1} \cdot l \cdot h^{-1}$	$g^{-1} \cdot l \cdot h^{-1}$	$g^{-1} \cdot l \cdot h^{-1}$
10	6	4.9	~ 0	0.18	0.17
18	10	10.1	~ 0	0.19	0.18
24	45	44.3	~ 0	0.18	0.19
34	100	~ 100	~ 0	0.14	0.17
40	200	~ 200	~ 0	0.13	0.18

TABLE 23. THE TREATABILITY OF FWA-3

Test duration	FWA-3 in the inflow	FWA-3 in the effluent	FWA-3 biodegradation coefficient K_b	Overall treatability coefficients	
				test unit K'_{COD}	control unit K_{COD}
days	c_o mg/l	c_e mg/l	$g^{-1} \cdot l \cdot h^{-1}$	$g^{-1} \cdot l \cdot h^{-1}$	$g^{-1} \cdot l \cdot h^{-1}$
5	5	3.0	0.029	0.18	0.17
11	10	6.2	0.021	0.15	0.19
18	16	8.1	0.042	0.14	0.19
24	24	18.0	0.012	0.06	0.18
32	80	64.5	0.008	0.05	0.18

TABLE 24. THE TREATABILITY OF FWA-4

Test duration	FWA-4 in the inflow	FWA-4 in the effluent	FWA-4 biodegradation coefficient K_b	Overall treatability coefficients	
				test unit K'_{COD}	control unit K_{COD}
days	c_o mg/l	c_e mg/l	$g^{-1} \cdot l \cdot h^{-1}$	$g^{-1} \cdot l \cdot h^{-1}$	$g^{-1} \cdot l \cdot h^{-1}$
11	4	4.3	~ 0	0.16	0.16
16	44	44.4	~ 0	0.15	0.15

TABLE 25. THE TREATABILITY OF FWA-5

Test duration	FWA-5 in the inflow	FWA-5 in the effluent	FWA-5 biodegradation coefficient K_b	Overall treatability coefficients	
				test unit K'_{COD}	control unit K_{COD}
days	c_o mg/l	c_e mg/l	$g^{-1} \cdot l \cdot h^{-1}$	$g^{-1} \cdot l \cdot h^{-1}$	$g^{-1} \cdot l \cdot h^{-1}$
11	4	4.2	~ 0	0.16	0.16
16	40	39.5	~ 0	0.16	0.15

Discussion

The obtained results show that FWA-2, FWA-4 and FWA-5 are not biodegraded in the activated sludge process. The highest removal rate was observed in the case of FWA-1 but even in this case the treatability coefficient K is rather low being equal to ca. 0.1 $g^{-1} \cdot l \cdot h^{-1}$. FWA-3 is less biodegradable than FWA-1; its K_b value is equal to ca. 0.03 $g^{-1} \cdot l \cdot h^{-1}$ and it decreases down to 0.01 when FWA-3 content in the inflow increases above 20 mg/l.

The overall treatability of wastewater is not affected significantly by any of FWAs in the inflow, except FWA-3 which decreases the K_{COD} value when its content in the inflow surpasses ca. 20 mg/l. Certain decrease of K_{COD} was observed also in the case of FWA-2 at its high content in the inflow (100 mg/l and above).

SECTION 8

THE RESULTS OF BIOASSAYS

METHYLETHYL KETONE, MEK

Behavior. The test organisms (fish, Lebistes reticulatus) reacted with darkening when they were exposed to MEK at the concentration of 2000 mg/1. At 2600 mg/1 MEK, the disturbing of the balance was observed. At 3400 mg/1 MEK, the fish were laying flat on the vessel bottom. At MEK concentrations near LC_{50}, i.e. 5000 mg/1, the fish were paralysed.

The mortality of fish at various MEK concentrations is shown in Fig. 66. LC_{50}-24 h was found as equal to 5700 mg/1 MEK.

The results of a supplementary test with algae Chlorella sp. are given in Table 26.

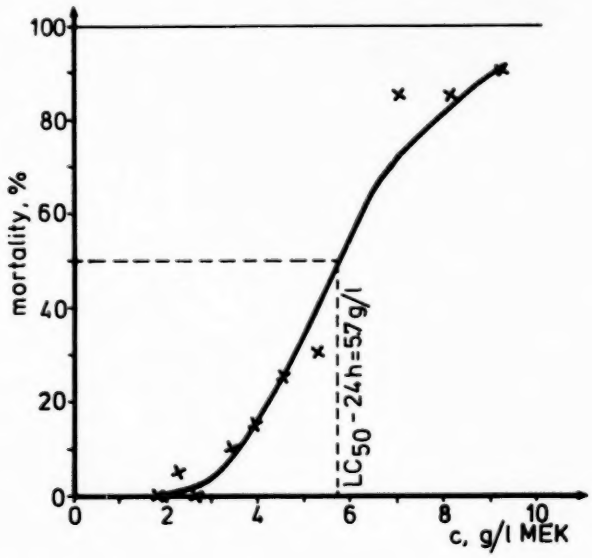


Figure 66. The mortality of fish at various MEK concentrations.

TABLE 26. CHLOROPHYLL "a" CONTENT IN THE CULTURE OF CHLORELLA AFTER 7 DAYS OF EXPOSURE TO THE PRESENCE OF MEK

Test no.	MEK content, mg/l	Chlorophyll "a" content, mg/l	
		blank culture	test culture
1	806	435	487
2	806	640	660

The differences in the chlorophyll content in the test samples and blank samples are within the range of analytical error. Therefore, any impact of MEK on the assimilation activity of Chlorella cannot be stated.

DIMETHYL AMINE, DMA

Behavior. After 20 min of exposure to the DMA at 64 mg/l, the fish reacted with fluttering movements. At 73 mg/l DMA the fish turn pale and their balance was disturbed (partial paralysis).

The mortalities of fish at various concentrations of DMA are presented in Table 27. From the results of the test (Table 27) the approximate value of LC_{50}- 24 h was calculated as equal to 55 mg/l.

TABLE 27. THE MORTALITY OF Lebistes reticulatus

Concentration mg/l \ Time	10 min	30 min	1 h	3 h	24 h
Blank	0	0	0	0	0
41.6	20	20	20	20	20
54.6	20	20	20	20	30
72.8	20	20	40	60	100
97.5	30	40	60	100	100
130.0	20	60	100	100	100

TABLE 28. CHLOROPHYLL "a" CONTENT IN THE CULTURE OF CHLORELLA AFTER 7 DAYS OF EXPOSURE TO DMA

Test no.	DMA content, mg/l	Chlorophyll "a" content, mg/l	
		blank culture	test culture
1	20	34	44
2	20	40	44
3	40	34	48
4	40	40	41
5	60	57	54
6	60	59	56

The test with algae Chlorella sp. indicates that DMA does not affect the assimilation activity of these algae. It is visible from the data presented in Table 28.

DIMETHYL FORMAMIDE, DMF

Behavior. The fish reacted with their balance being disturbed within the first hour of exposure to 650 mg/l DMF. After 24 hours, fish turned dark. At 750 mg/l DMF, the lateral or ventral bend of the fish body was observed. The 870 mg/l DMF caused fluttering movements of the fish.

The mortality data are presented in Fig. 67. The value of LC_{50}- 24 h was found to be 1300 mg/l.

Supplementary test with algae Chlorella did not indicate any affect of DMF on the assimilation activity of Chlorella. The results of this test are given in Table 29.

PARA-NITROPHENOL, PNP

Behavior. At the lower PNP concentration the reaction of fish was not significant. At 18 mg/l PNP the animals moved slowly at the bottom and after 30 min they turned somewhat pale and the first symptoms of partial paralysis were observed. At the concentration of 32 mg/l, after 20 minutes the fish turned on their side (they looked almost not alive except for the weak action of heart and sporadic movements of the fin). After 2 or 3 hours all organisms were dead and dropped to the bottom. The mortality of fish at various PNP concentrations is marked on Fig. 68. The LC_{50}- 96 h was found as equal to 19 mg/l.

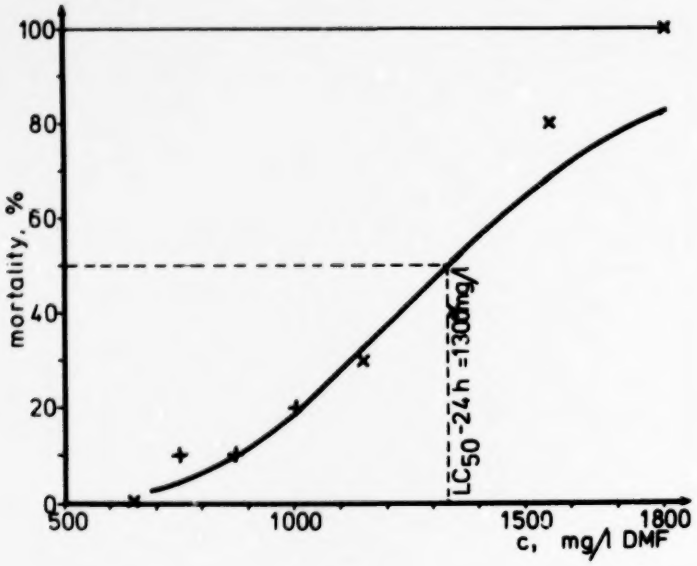


Figure 67. The mortality of fish at various DMF concentrations.

TABLE 29. THE CHLOROPHYLL "a" CONTENT IN THE CULTURE OF CHLORELLA AFTER 7 DAYS OF EXPOSURE TO DMF

Test no.	DMF content, mg/l	Chlorophyll "a" content, mg/l	
		blank culture	test culture
1	945	57	46
2	945	40	46
3	1890	34	43
4	1890	57	50

ORTHO-CHLOROPHENOL, OCP

Behavior. Within the first 10 minutes in all solutions tested, the movements of fish were rapid and violent. At lower concentrations the fish were acclimated to OCP presence after one hour and their behavior became the same as in the control flask. At the concentration of 15 mg/l and higher most of the fish were paralysed after one hour. The others got together at the bottom. At the highest concentration and after one or two hours, the

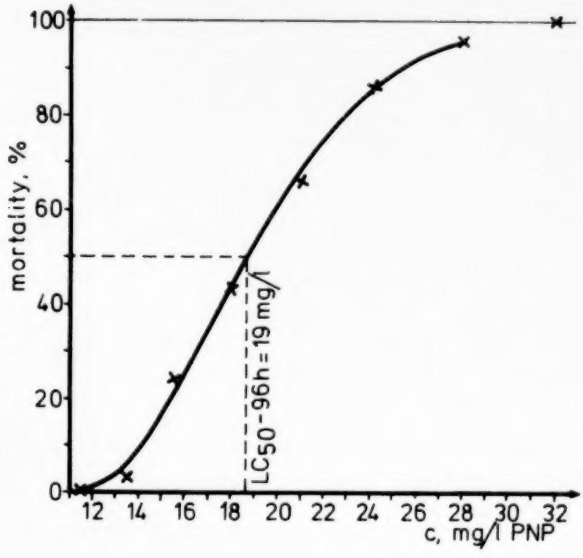


Figure 68. The mortality of fish at various PNP concentrations.

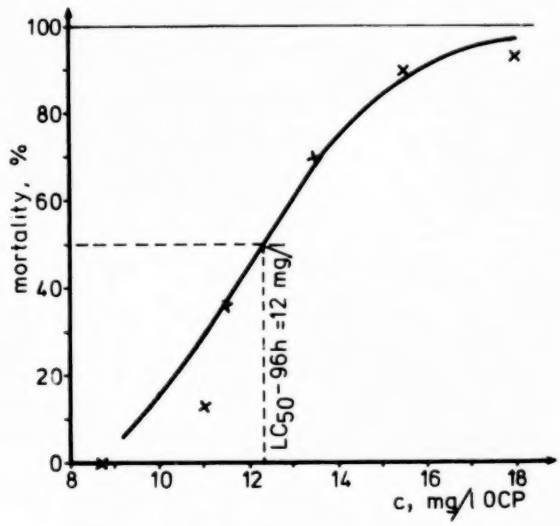


Figure 69. The mortality of fish at various OCP concentrations.

fish were almost completely paralysed. Several of them were alive in this state during one day.

Mortality of the fish at various OCP concentrations is plotted in Fig. 69. The calculated LC_{50}- 96 h was 12 mg/1.

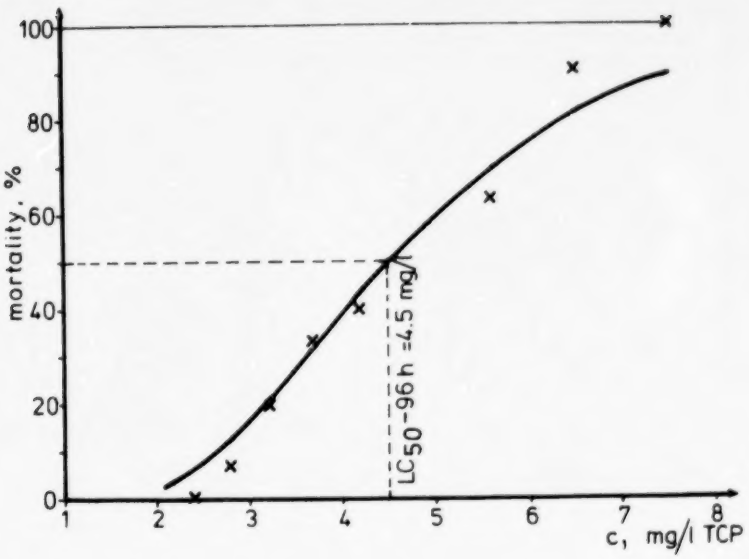


Figure 70. The mortality of fish at various TCP concentrations.

TRICHLOROPHENOL, TCP

Behavior. Four or five minutes after the fish were placed in the test flasks the solutions in the flasks became turbid. After the next 5 or 10 minutes the animals began to move rapidly; later on their movements became slower and the fish kept themselves near the surface. After 30 to 40 minutes from the beginning of the experiment, a fine precipitate, causing turbidity, settled so that the solution became clear. This precipitate, agglomerated with fish slime, was visible on the mouths, gill covers and fins of the fish. At the concentrations above 4.9 mg/l most of the fish were paralysed.

Mortality of fish in the presence of TCP is shown in Fig. 70. The interpolated LC_{50}- 96 h value was 4.5 mg/l.

DICHLORODIETHYL ETHER, DCDEE

Behavior. After 20 to 30 minutes the animals turned slightly dark and their colors became more grayish. At lower concentrations, the fish got together at the surface, moving slowly. At higher concentrations the symptoms of paralysis were visible after 1.5 hours: the fish kept abnormal positions. The dead animals floated at the surface.

Mortality is presented in Fig. 71. The calculated LC_{50}- 96 h value equals 190 mg/l.

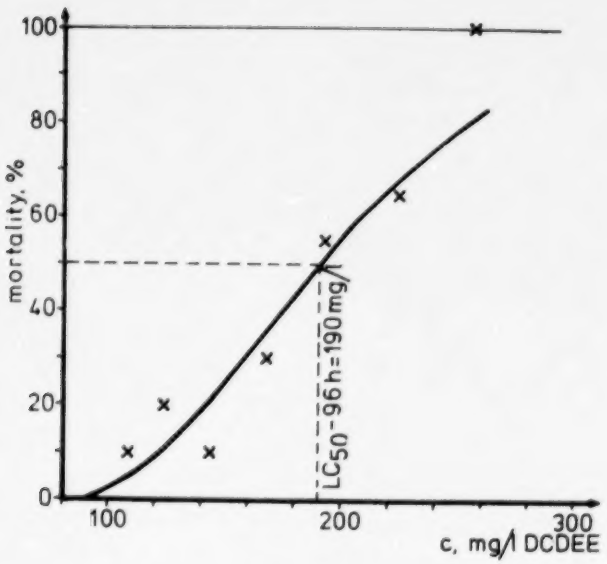


Figure 71. The mortality of fish at various DCDEE concentrations.

FLUORESCENT WHITENING AGENTS, FWAs

FWA-1

Behavior. During the first period of the test the fish reacted to the FWA-1 presence with rapid movements. After several minutes the fish were posed on the bottom of the vessel rapidly moving with their chest fins. In the test media with the highest FWA-1 content (21 - 37 mg/1), the fish were floating, turned with their backs down, and almost did not react when touched. At FWA-1 content of 15.5 mg/1 the fish were partially paralysed.

The mortality of fish is presented in Fig. 72. LC_{50}- 96 h was equal to 16 mg/1. The determination of LC_{50}- 24 h to Daphnia magna gave the result of 23 mg/1.

FWA-2

Behavior. At the lower FWA-2 content (below 1 g/1) the fish were animated during the first minutes of the test, and then they moved more slowly. After 24 hours of the test, the behavior and the look of the fish were normal. At the highest tested concentration (above 3 g/1), paralysis and death of fish were observed. Extravasations were visible on various parts of the fish body.

The mortality of fish is presented in Fig. 73. LC_{50}- 96 h for Lebistes reticulatus was equal to 1760 mg/1. The LC_{50}- 24 h of FWA-2 for Daphnia magna was found to be 3900 mg/1.

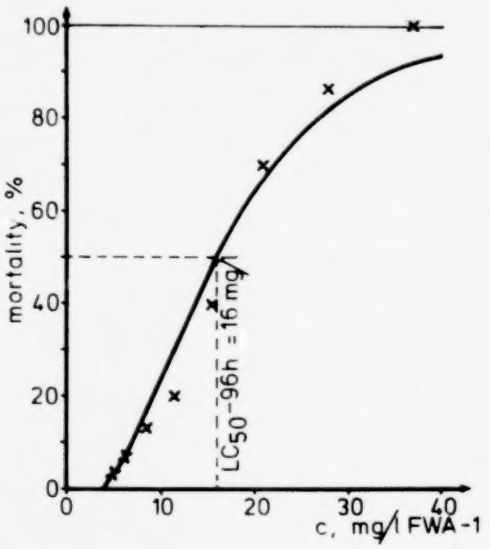


Figure 72. The mortality of fish at various FWA-1 concentrations.

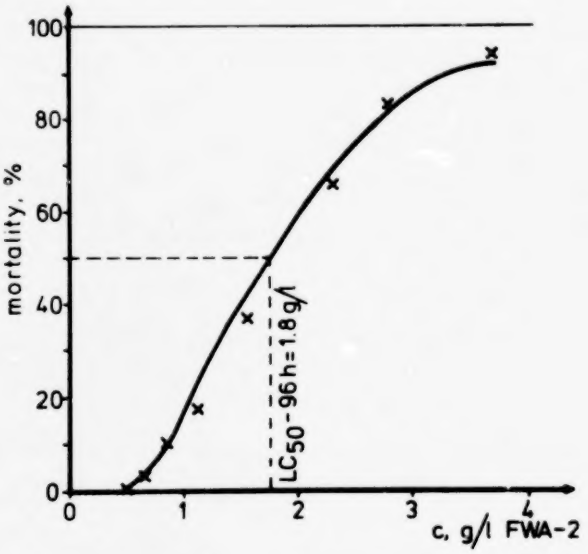


Figure 73. The mortality of fish at various FWA-2 concentrations.

FWA-3

Behavior. During the initial three days of the test, in the solutions with ca. 100 mg/l FWA-3, the fish were sluggish but reacted when touched. During the fourth day, the same symptom was visible in the fish exposed

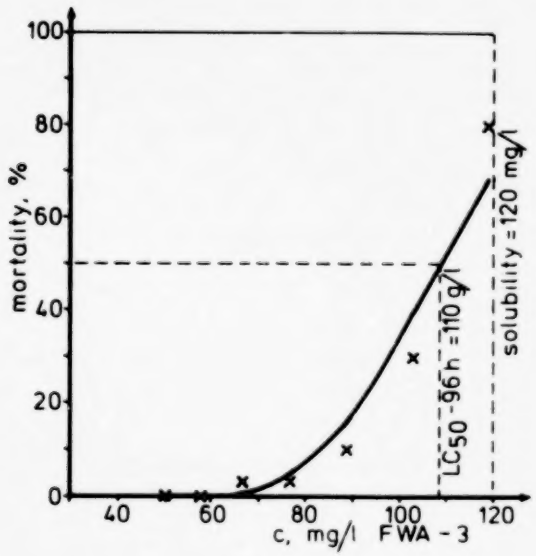


Figure 74. The mortality of fish at various FWA-3 concentrations.

to 89 mg/l FWA-3. At the FWA-3 concentration of 120 mg/l, little extravasations on the fish body were visible.

The mortality of fish is presented in Fig. 74. The LC_{50}- 96 h of Lebistes reticulatus was equal to 110 mg/l. The LC_{50}- 24 h of Daphnia magna surpassed the solubility of FWA-3 in water. At the saturated FWA-3 solution, the mortality of Daphnia magna amounted to 20 percent.

FWA-4

Behavior. At FWA-4 content of 4 to 10 g/l, the fish were paralysed and the extravasations were visible near the gills of the fish. The backs of the dead fish were bent near the caudal fins.

The mortality of the fish is presented in Fig. 75. The LC_{50}- 96 h of Lebistes reticulatus was 3000 mg/l. The LC_{50}- 24 h to Daphnia magna equaled 9400 mg/l.

FWA-5

Behavior. At the lower FWA-5 concentration (4.5 g/l) the fish were sluggish but reacted when touched. After several hours their behavior was quite normal. At the higher FWA-5 concentration (6 - 7 g/l), the fish were paralysed and extravasations were visible. The skin of the dead fish was chapped.

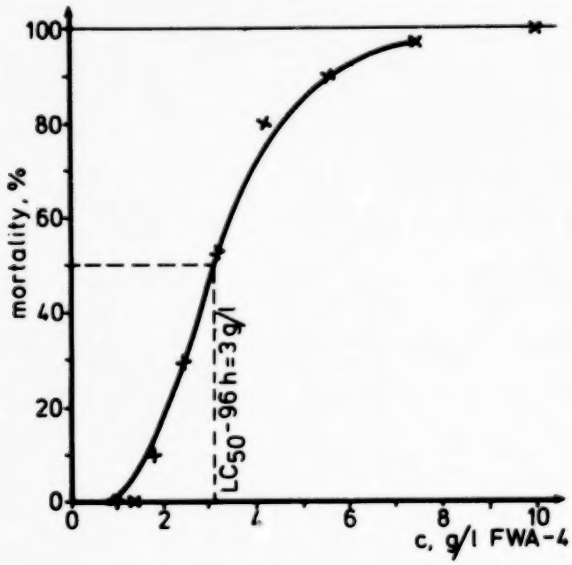


Figure 75. The mortality of fish at various FWA-4 concentrations.

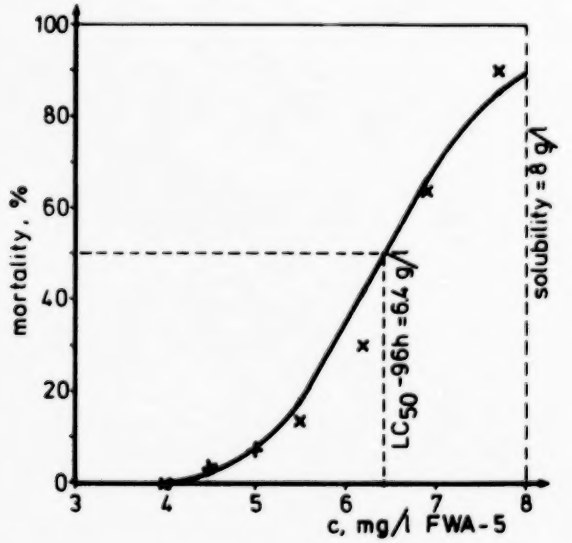


Figure 76. The mortality of fish at various FWA 5 concentrations.

The mortality of fish is presented in Fig. 76. The LC_{50}- 96 h of Lebistes reticulatus was 6400 mg/l. The LC_{50}- 24 h of Daphnia magna was 5600 mg/l.

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